BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING AT THE JOHNS HOPKINS UNIVERSITY APPLIED PHYSICS LABORATORY ANNUAL REPORT OCTOBER 1, 1975 - SEPTEMBER 30, 1976

JOHNS HOPKINS UNIVERSITY
LAUREL, MARYLAND

SEPTEMBER 1976

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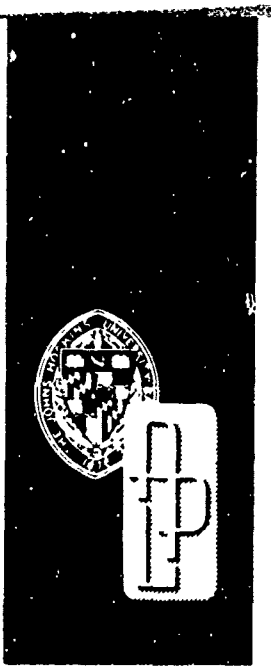

Annual Report

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING

at The Johns Hopkins University Applied Physics Laboratory

OCTOBER 1, 1975–SEPTEMBER 30, 1976

THE JOHNS HOPKINS UNIVERSITY ■ APPLIED PHYSICS LABORATORY

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The School of Medicine, the School of Hygiene and Public Health, and The Johns Hopkins University Applied Physics Laboratory have developed a collaborative program of biomedical research and development over the past several years. An important objective of the program is to apply the expertise in engineering and physical sciences, and systems analysis acquired in defense and space research and development by APL to problems of medical research and health care delivery. At the present time this program has grown to include collaboration with nearly all of the clinical departments and with several of the basic science departments of the medical divisions. Currently active programs exist in ophthalmology, neuro-sensory research and instrumentation development, cardiovascular systems, radiology, prosthetic systems, computer support, and clinical engineering.

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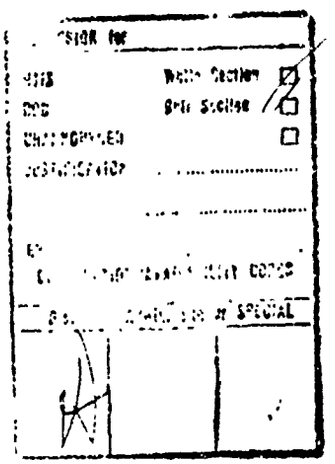

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THE JOHNS HOPKINS UNIVERSITY ■ APPLIED PHYSICS LABORATORY
Johns Hopkins Road ○ Laurel, Maryland ○ 20810
Operating under Contract N00017-72-C-4401 with the Department of the Navy

FOREWORD

The School of Medicine, the School of Hygiene and Public Health, and The Johns Hopkins University Applied Physics Laboratory have developed a collaborative program of biomedical research and development over the past several years. An important objective of the program is to apply the expertise in engineering, the physical sciences, and systems analysis acquired in defense and space research and development by APL to problems of medical research and health care delivery. At the present time this program has grown to include collaboration with nearly all of the clinical departments and with several of the basic science departments of the medical divisions. More recently a collaborative program in clinical engineering has been established with the Johns Hopkins Hospital. Currently active programs exist in Ophthalmology, Neurosensory Research and Instrumentation Development, Cardiovascular Systems, Radiology, Prosthetic Systems, Computer Support, and Clinical Engineering. This application of "state-of-the-art" technology has contributed to advances in many areas of basic medical research and in clinical diagnosis and therapy through improvement of instrumentation, techniques, and basic understanding.

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BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING §35
Research by: RMP
Support: USPHS Grant GM-21899-02 (NIGMS) and APL
B. F. Kim and J. Bohandy
April-June 1976

HIGH-RESOLUTION SPECTROSCOPY OF PORPHYRINS

This project is studying the energy-level structure of porphyrin compounds by electron spin resonance (ESR) and optical spectroscopy. Porphyrin molecules form the basis for several larger molecules (such as hemoglobin and chlorophyll) that are important in many biological processes. Detailed knowledge of the quantum structure of porphyrins, obtained from spectroscopic studies, should contribute to a fundamental understanding of their roles in chemical and biological processes. Special techniques are used in this study to obtain spectra with high resolution, thereby allowing the energy level structure to be obtained in greater detail than is possible with conventional techniques.

BACKGROUND

Porphyrin molecules have a tetrapyrrole structure that can have two hydrogen atoms in the interior of the ring (called the free base) or the hydrogen atoms can be replaced by a single metal atom to form various metalloporphyrins (see Fig. 1). Single crystals of the aromatic host, triphenylene, into which small amounts of free base and metalloporphins were incorporated as guest molecules, have been grown from solution. The use of a periodic lattice eliminates inhomogeneous broadening of the spectral lines, while working at 4.2°K greatly reduces thermal broadening. In this manner, sharp-line optical spectra of porphyrins have been obtained. The optical spectra give information on the excited electronic states. If the metal atom in the center of the porphyrin is a paramagnetic material (such as vanadium or copper), ESR techniques may be used to study the ground state and local environment of the paramagnetic species. When single crystal samples are used, the number of site species and the orientations of the molecules in the host can be determined. An ESR study of copper porphin (CuP) was undertaken to complement the optical data and previous ESR spectra of vanadyl porphin.

Fig. 1 Molecular Structure of Free Base Porphin and Metalloporphins. M represents a metal ion. (76-3/50)

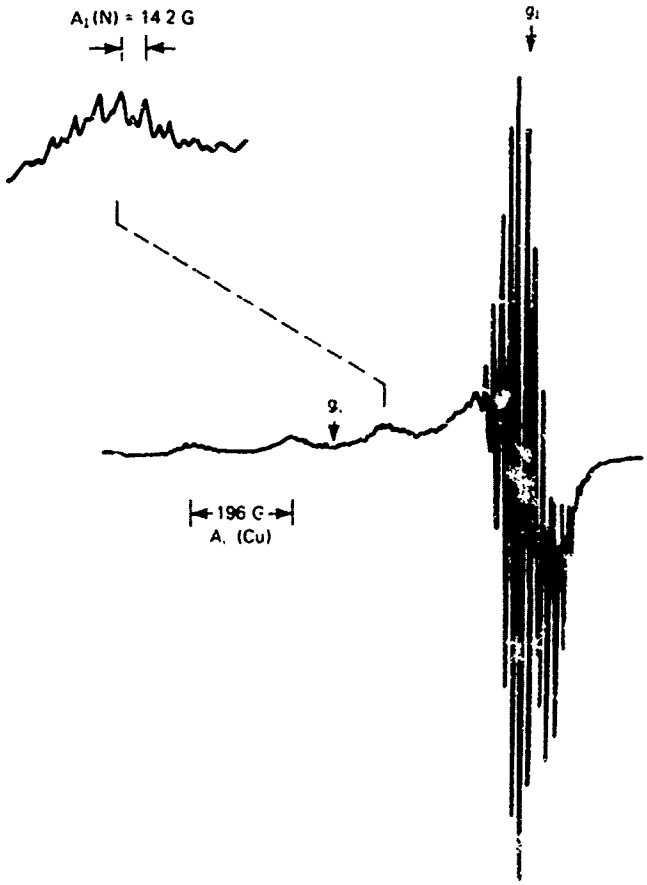

Fig. 2 ESR Powder Spectrum of CuP in Triphenylene at 16.449 GHz. The magnetic field is increasing to the right. (76-3/51)

SUMMARY

The ESR spectra can be characterized by a g-factor or electron magnetic moment and, since Cu^{2+} has a nuclear spin, by the magnetic interaction between the electron and the copper nucleus. The latter interaction is described by the hyperfine coupling constant, A, and results in a splitting of the ESR line into four hyperfine lines. In this case, another interaction manifests itself in the experimental data. There is an interaction, A_N, between the electron and the nuclei of the four-nearest-neighbor nitrogen atoms surrounding the Cu^{2+} ion. Since the magnetic electron is localized mainly on the Cu ion, this interaction is called a superhyperfine (shf) interaction. If all the nitrogens are equivalent, one would expect each of the four Cu hyperfine lines to be split into nine shf components. Triphenylene has four molecules per unit cell, and ESR spectra from CuP in each nonequivalent site will be observed. One sees that, in general, the spectrum will be extremely complex. At the

expense of the orientational information, the multiple site problem can be eliminated by crushing single crystals and obtaining a so-called powder ESR spectrum. The principal components of the g, A, and A_N tensors can be determined from one powder spectrum. An ESR powder spectrum obtained at Ku-band is shown in Fig. 2. Three parallel components are resolved with the fourth just masked by the perpendicular spectrum. Each parallel hyperfine line is further split by the shf interaction into lines separated by 14.2 G. In addition, there are lines that are approximately one-half as intense in between the shf lines. These are due to either a magnetically inequivalent site or the resolution of lines from the ^{65}Cu isotope. On the basis of their expected intensity ratio of approximately two-to-one and because the weaker lines appear halfway between the stronger ones at two different microwave frequencies, these weaker lines are assigned to ^{65}Cu.

The perpendicular portion of the powder spectrum is more complex, due not only to the shf structure but also to the larger anisotropy in the nuclear hyperfine interaction. Although the isotope splittings in the perpendicular region were not observed at room temperature, spectra taken at low temperatures did exhibit structure attributed to ^{65}Cu.

Analysis of the data showed the tensors to be axially symmetric. The resulting principal components are

$g_\parallel = 2.190$ $A_\parallel(Cu) = 196$ G $A_\parallel(N) = 19.8$ G

$g_\perp = 2.043$ $A_\perp(Cu) = 33$ G $A_\perp(N) = 14.2$ G

Instead of performing a complete rotational study of the single crystal samples, it was decided to see if the symmetry directions and principal axis directions were the same as were found for vanadyl porphin in triphenylene (Ref. 1). This was found to be the case, with the normals to the four porphin planes making equal angles of 51° with the c-axis. Weaker lines attributed to ^{65}Cu also were observed in the parallel single crystal spectrum.

There are several orientations of the crystal for which the magnetic field is perpendicular to at least one of the normals to the porphin planes. It was not possible to isolate completely the perpendicular spectrum of one site from overlapping lines due to other sites. Furthermore, when $A_\parallel(N)$ does not equal $A_\perp(N)$, there is ϕ dependence of the shf splitting and more than nine lines for each Cu hf line may be observed. These factors made the analysis of the perpendicular spectrum difficult, but the spin-Hamiltonian parameters obtained were in good agreement with the powder data. The single crystal data are consistent with CuP molecules in substitutional triphenylene sites.

FUTURE PLANS

Interpretation of the optical spectra requires a knowledge of the orientation of the porphyrin planes in the crystal. For complete axial symmetry, only the orientation of the normals can be fixed from the ESR data. The angular dependence of the shf interaction in CuP permits the absolute orientation of the plane to be determined in principle. In practice, however, overlapping spectra from different sites made this very difficult. Therefore, a search for a crystalline host with one molecule per unit cell will be made. Sharp line selective excitation optical spectroscopy using a dye laser will be used to eliminate the multiple site problem for those hosts having multiple inequivalent sites.

REFERENCE

1. J. Bohandy, B. F. Kim, and C. K. Jen, J. Mag. Resonance, Vol. 15, 1974, p. 420.

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING §36
Research by: RTP
Support: USPHS Grant EY-01019 (NEI)
R. A. Farrell, B. F. Kim, and R. L. McCally
October 1975-September 1976

CORNEAL STRUCTURE

The general goal of this research is to determine the structural basis of corneal function. The behavior of the cornea is necessarily determined by its molecular structure. Thus the organization of the corneal macromolecules and the dependence of physiological behavior on that organization are of considerable importance in connection with the behavior of a healthy cornea and the causes and possible control of diseased corneas.

SUMMARY

Our previous studies (Refs. 1 through 3) have shown that light-scattering measurements afford a noninvasive tool for studying corneal structure. During this reporting period, the construction of a low-angle light-scattering apparatus was completed. The design of the apparatus is based on diffraction-limited optics; measurements (Ref. 4) of the depth-resolving power of the apparatus at various angles prove that it will meet our requirements. Also, a program has been initiated to determine the effects of transcorneal pressure on the small-angle light-scattering patterns observed when the cornea is illuminated by collimated polarized light. Additionally, we have reviewed models of corneal structure in light of our previous transmissivity measurements and electron micrographs (Ref. 5).

DISCUSSION

It is generally recognized that the transparency of corneas of normal thickness is a consequence of interference effects caused by an ordering in the spatial arrangement of the constituent collagen fibrils about one another. Corneas swollen by storage in cold Ringer solution are less transparent. Electron micrographs (EM's) of swollen corneas show that the ordered fibril arrangement has been disrupted and that the amount of disruption appears to increase markedly with depth into the swollen cornea. Previous studies have indicated that the structures depicted in these EM's would be expected to have their largest effect on light scattering at small scattering angles. Consequently we have designed an apparatus with an angular resolution of ±1° to measure light scattered by the cornea into angles from 6° through 15° while resolving the depth of the scattering volume to ≤ 200 μm (approximately one-third the swollen corneal thickness).

The ultimate depth resolution is determined by physical and geometrical limitations inherent in the measurement process (these limitations are discussed in Ref. 4). The depth-resolution requirement for light scattering by the cornea at a scattering angle of 6° is close to the theoretical limit for such measurements; hence an apparatus is required that has an optical response free of aberrations, limited only by diffraction effects. Such an apparatus has been designed and constructed. Tests of its depth resolution, using a thin scatterer, gave results within the specified limit (200 μm) for corneal light scattering (Ref. 4).

Additional constraints on the design of the light-scattering apparatus are imposed by physiological considerations when observing light scattering from an excised cornea. In order to maintain the cornea in a reasonably natural state, the cornea must be totally immersed in a Ringer solution, and the proper physiological pressure applied to the cornea. The optical interface between the vessel that contains the cornea and Ringer solution and the surrounding air will cause refraction of the incident and scattered light beams, thus (in general) causing misalignment of the apparatus. Moreover, refraction is wavelength dependent, so that in order to achieve experimental results within the specified precision, separate realignment of the optical components would be necessary for each wavelength band of interest. These effects can be avoided, however, if the incident and scattered beam directions are normal to the fluid-glass-air interfaces. This configuration can be achieved by placing the scattering volume at the center of curvature of two spherical curved windows, as shown schematically in Fig. 1. A sample vessel incorporating this feature is presently under construction.

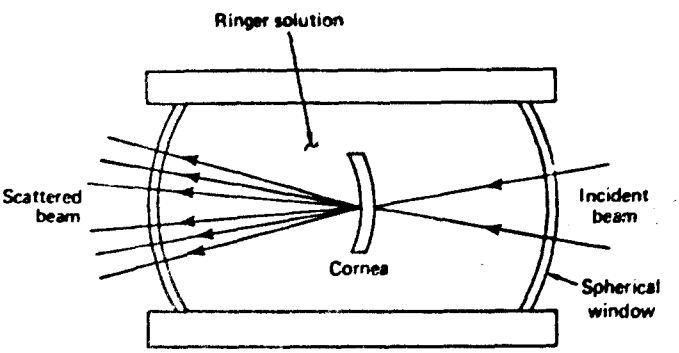

Fig. 1 Cornea Light-Scattering Sample Cell. (76-3/47)

A second type of small-angle light-scattering experiment (SALS) has been performed. In SALS, the cornea is illuminated with a collimated beam of polarized light from a laser. Light scattered with polarizations either parallel or perpendicular to that of the incident beam is observed at scattering angles of less than 5°. These scattered light intensities form patterns that are called the $I_{\parallel}$ and the $I_{\perp}$ patterns, respectively. The $I_{\parallel}$ scattering results primarily from isotropic optical density fluctuations within the scatterer, whereas the $I_{\perp}$ scattering is caused by anisotropic optical density fluctuations (Ref. 6). Other researchers have attempted to elucidate corneal structure using SALS (Refs. 6 and 7); however, reconciliation of the observed scattering patterns with model structures for which scattering patterns are known and with structures suspected histologically has been equivocal. A possible difficulty is that these measurements were not made under physiological conditions with the intraocular pressure maintained. Corneal lamellae appear wavy in EM's prepared by standard techniques; however, it has been suggested by J. Francois (private communication) that fixation of the cornea under its normal intraocular pressure may reduce the wave amplitude. We are presently working to confirm that the waviness of the lamellae is reduced when the cornea is fixed under pressure.

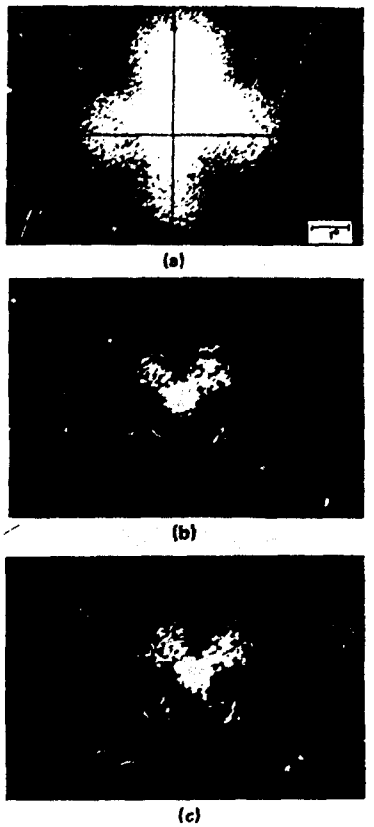

Fig. 2 The Effect of Increasing Transcorneal Pressure on the I+ Scattering Pattern. (a), (b), and (c) are a 0, 9, and 18 mm Hg, respectively. The arrows show the direction of the polarizer axes. The scale for the true scattering angle is given in (a); the photographs were processed identically and thus are at the same scale. (76-3/65)

Structures of dimensions comparable to the periodicity of these waves would be expected to affect SALS measurements; consequently, we have measured SALS from the central region of rabbit corneas as a function of intraocular pressure (cf Fig. 2). The effects of birefringence on these measurements were removed by standard means (Refs. 6 and 7). I_+ scattering patterns in particular are changed markedly by application of intraocular pressure. Zero-pressure patterns are consistent with those in Ref. 6. At increased pressure, the zero-pressure pattern persists but with reduced intensity, and new features appear at smaller scattering angles. We are presently working on the structural interpretation of the data in terms of cellular and fibrillar structures shown by standard histological techniques. In any event, future SALS investigations on corneas must account for these observations.

The nature of the forces that cause the spatially ordered fibril arrangement is of obvious importance, and we have discussed the constraints that our light-scattering measurements impose on models of corneal structures (Ref. 5). Recently, Twersky (Ref. 8) proposed a modified hard-core model in which the collagen fibrils are coated by a substance having the same refractive index as the corneal ground substance. This coating provides an effective hard "statistical mechanical" particle whose radius is larger than the optical radius of the scattering particle. Short-range correlations are thereby introduced in the fibril positions because two particles cannot approach each other closer than this distance. Although the hard-core diameter needed to account for transparency in this model is somewhat greater than the distances of nearest approach shown in EM's, the short-range nature of the correlation does lead to the observed dependence of scattering on wavelength. (Scattering calculations based on the structures shown in EM's lead to this same wavelength dependence.) Most previous models led to long-range correlation and therefore to a different dependence of scattering on wavelength.

Twersky accounts for the effect of corneal swelling by an increase in the space available for each particle, causing a loss in the regularity of their positions and a consequent decrease of destructive interference. Although the model predicts a transmissivity at 500 nm, which is in qualitative agreement with experiments on corneas swollen to 1.5 times their initial thickness, the general disorganization of fibril positions predicted by this model does not lead to the measured variation of transmissivity with wavelength. EM's of swollen corneal stromas show the presence of large voids in the fibril arrangement. The presence of these "lakes" would cause the observed variation of scattering with wavelength. Our study of Twersky's and other structural models shows that a model that elucidates the underlying mechanisms responsible for the short-range ordering of fibrils in normal corneas and for the structures (such as lakes) that give rise to the observed wavelength dependence of scattering in swollen corneas remains a cherished goal.

FUTURE PLANS

The new apparatus will be used to measure the depth dependence of corneal scattering at small scattering angles. Calculations of scattering one would expect from wavy lamellae and from cells will be initiated.

REFERENCES

1. R. A. Farrell, R. L. McCally, and P. E. R. Tatham, "Wave-Length Dependencies of Light Scattering in Normal and Cold Swollen Rabbit Corneas and their Structural Implications," J. Physiol., Vol. 233, 1973, pp. 589-612.
2. R. A. Farrell and R. L. McCally, "On the Interpretation of Depth Dependent Light Scattering Measurements in Normal Corneas," Acta Ophthal., Vol. 54, 1976, pp. 261-270.
3. R. L. McCally and R. A. Farrell, "The Depth Dependence of Light Scattering from the Normal Rabbit Cornea," Exptl. Eye Res., Vol. 23, 1976, pp. 69-81.
4. B. F. Kim, "Low Angle Light Scattering Apparatus" (to be published in Rev. Sci. Instrum.).
5. R. A. Farrell and R. L. McCally, "On Corneal Transparency and Its Loss with Swelling," J. Opt. Soc. Am., Vol. 66, 1976, pp. 342-345.
6. E. P. Chang, D. A. Keedy, and J. C. W. Chien, "Ultrastructures of Rabbit Corneal Stroma: Mapping of Optical and Morphological Anisotropies," Biochim., Biophys. Acta, Vol. 343, 1974, pp. 615-626.
7. F. A. Bettelheim and D. Kaplan, "Small Angle Light Scattering of Bovine Cornea as Affected by Birefringence," Biochim. Biophys. Acta, Vol. 313, 1973, pp. 268-276.
8. V. Twersky, "Transparency of Pair-Correlated, Random Distributions of Small Scatterers, with Applications to the Cornea," J. Opt. Soc. Am., Vol. 65, 1975, pp. 524-530.

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING §37
Research by: REM
Support: NIH Grant EY-01312 (NEI)
B. F. Hochheimer (APL) and J. L. Calkins (JHMI)
October 1975-September 1976

HOLOGRAPHIC STRESS TESTING OF THE SURGICAL CORNEA

The objective of this work is to design a prototype holographic camera and recording methodology suitable for performing double-pulsed holographic interferometry on the cornea. Such a camera would provide a noninvasive, objective technique for determining in vivo the state of healing and the tensile strength of postoperative corneal wounds. The system should be sensitive enough to rely solely on ocular pulse pressure as the applied stress. The system should be safe and should have sufficient stability to allow its use in a clinical setting without requiring general or retrobulbar anesthesia. Techniques already developed will be refined and optimized. Rabbit keratoplasties will provide calibration and histologic confirmation of the holographic interferograms obtained from animals and patients.

This method may be capable of providing fast, objective data on (a) metabolic or pharmacologic agents that promote or retard wound healing; (b) types of sutures, incisions, and suturing techniques for optimal mechanical stability; and (c) corneal graft rejection and its early detection.

Techniques for clinically measuring and objectively assessing corneal wound healing are scarce. No single criterion is reliable. For animal studies, one can measure wound strength very objectively by forcibly pulling apart the edges of the healed wound and measuring the force necessary to do so. Of course, this destroys the specimen and is not suitable for clinical application.

Our proposed technique of holographic stress testing serves as an objective measurement of wound strength that, we now believe, will be feasible for clinical application. The technique is not only nondestructive, it is also noninvasive. Intraocular pressure variations that closely follow each heartbeat are sufficient to serve as the stressing force; no direct instrumentation or external force needs to be applied.

The technique of holographic interferometry for nondestructive testing is used in industry as an extremely sensitive means of detecting structural imperfections and weaknesses. It can be applied to a wide variety of finished products such as turbine blades or automobile tires without having to destroy the sample being tested.

The optical interferometry technique is a very sensitive detector of small deformations. A hologram records light phase and amplitude and can therefore retain sensitivity to a fraction of a wavelength of light. Optical wavelengths are about 0.5 μm which permits very high-resolution detection of these deformations.

SUMMARY AND DISCUSSION

We have successfully demonstrated that holographic interferometry is an extremely sensitive test for the noninvasive, nondestructive stress testing of corneal wounds in several postoperative patients. The only stress used on the cornea is that supplied by the ocular pulse pressure following each heartbeat. One patient had had a successful penetrating keratoplasty six years previously (for keratoconus) and was, for all intents and purposes, fully healed. Yet the hologram revealed bulging of the corneal wound, implying that the wound was not up to 100% strength. This is substantiated to some extent by cases in the literature where, from two to eight years postoperatively, blunt trauma has caused dehiscence along the graft wound margin.

In designing and constructing our holographic camera to carry out this task, several technical obstacles were encountered that are discussed below.

Stability. Since holography requires extreme stability during a given exposure for good image quality, the major concern has always been that the patient could not hold still long enough. Of course, by making a short exposure, practically any moving object can be successfully recorded. But the only way to do this (and still avoid using double-pulsed solid-state lasers because of both their frequency shift between pulses and their higher cost) was to greatly increase either the laser power or our recording efficiency. The former route poses safety problems regarding retinal exposure, so we had no choice but to optimize our light-collection efficiency. Our efficiency was so poor that it took a 20-s exposure to get a hologram of a rabbit cornea (using our 50 mW He-Ne laser). Over 20 s, even though the eye was enucleated and suspended in a rigid hydraulic system, there was enough evaporation and tissue movement to cause many artifact fringes across the field. An even more serious drawback to our simple technique of diffusely illuminating the cornea was that the coherence was apparently broken up by multiple internal reflections within the corneal stroma.

Illumination. The system we finally devised has the advantage of being both extremely efficient and very simple. It solves the paradox in our requirement; that is, a diffuse illumination was desired since it would provide the large spatial bandwidth required to fully record very irregular or nonuniform corneas (such as post-operative keratoplasties), yet we wished to have all the advantages of specular reflection, such as high reflectivity and negligible loss of temporal coherence. Our first successful version was a hemispherical diffuse reflector (similar to a Goldmann perimeter dome), with the eye placed at the center. The dome was uni-

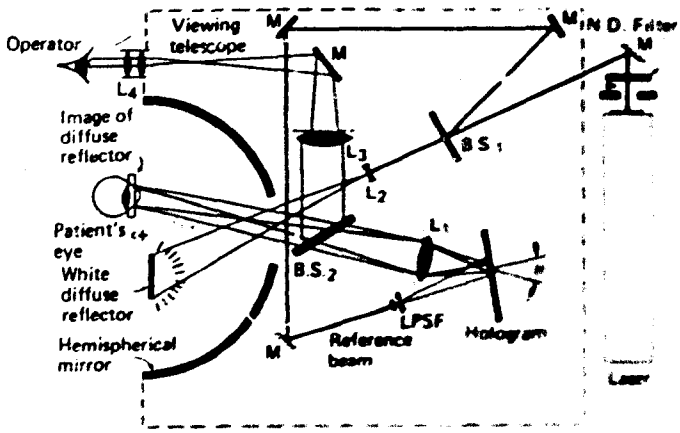

Fig. 1 Holographic Camera. (188761)

formly illuminated by laser light during the exposure, and the hologram was recorded through a hole centered in the dome. This worked beautifully insofar as recording quality was concerned, yet we were still throwing away 99% of our light because of Lambertian scattering within the dome. (Multiple scattering did not help us there as it would in a visual field plotter where it contributes to increased background luminance. Here, any multiple reflection destroys coherence and thus is dealt with as "noise.")

Our final version, which actually makes the whole project feasible, consists of a small diffuse reflector about 12 mm in diameter that is positioned on the patient's forehead as he places his head into a hemispherical mirror in much the same way a patient is positioned in a perimeter. All of the laser light (except the small amount tapped off for the reference beam) can be very effectively pumped onto this small diffuser. Virtually all the light is then collected by the hemisphere and reimaged back onto the cornea. The light is specularly reflected by the cornea onto the holographic plate. Two fringe benefits of this method are that completely uniform illumination is apparent when viewing the reconstructed holographic image, and displacement sensitivity vectors are perpendicular to the surface over the entire cornea.

Primarily as a result of the extreme efficiency of this illuminating system, we are able to take 8-ms exposures (using only 50 mW of laser output); this produces only 1/10 to 1/100 the amount of retinal irradiance per exposure as that received during a conventional fundus photograph. We would like to reduce the exposure to 1 ms or less; therefore, we have ordered a krypton ion laser with a 500-mW output. We have demonstrated that from 1- to 10-ms exposure time provides adequate stability, even for a patient in a clinical setting.

Fringe Control. The hologram is synchronized with an ECG monitor connected to the patient via two wrist leads. This allows a double exposure to be made during the linear phase of increasing intraocular pressure (usually about 0.2 s after the R-wave trigger signal). To increase the stressing pressure, ΔP, we simply separate the two pulses by a longer interval while keeping within the linear rise. Unfortunately, the patient may move his eye slightly during this interval. (Δt is typically 0.06 s.) Such movement does not affect stability per se, since each exposure is only about 1 ms. However, the movement does constitute "rigid body motion" and is depicted very accurately in the final fringe pattern. By having the rigid body motion fringes superimposed on the stress-deformity fringes, interpretation of the latter becomes more difficult. Since such rigid body motion (translation or rotation) produces primarily either linear or quadratic phase errors in the wavefront, they can theoretically be canceled out by reference beam manipulation at the time of reconstruction (provided a dual-reference beam technique is used). Large amounts of movement have been negated in this manner when studying inanimate objects. It would be a great advantage if applicable, since most patients seem to exhibit some degree of "eye movement artifact."

FUTURE PLANS

A holographic camera (Fig. 1) is being constructed using a krypton laser in place of the helium-neon laser previously used. The krypton laser has a power output of 500 mW compared to the helium-neon output of 50 mW. With the extra power, the exposure time can be considerably shortened to reduce the stability problem.

We are also constructing a "patient holder" so that the patients can be positioned accurately and securely by the camera operator. Fringes caused by movement of the patient should be eliminated with this device.

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING §38
Research by: FSE
Support: USPHS Grant EY01178 (NEI)
L. J. Viernstein (APL) and I. P. Pollack (JHMI)
October 1975-September 1976

CONSTANT PRESSURE TONOGRAPHY

The purpose of this research is to evaluate a new method of tonography for the differential diagnosis of glaucoma. Tonography is a technique for measuring the ease (facility) by which aqueous fluid leaves the interior of the eye and enters the venous system. Blockage of the aqueous outflow channels of the eye is a cause of glaucoma and also of the accompanying ocular hypertension that leads to blindness. In the new tonographic method, an applanating surface flattens the cornea until a prescribed level of intraocular pressure is reached. This pressure is maintained constant by servo control of the applanation force. The increase in force required to hold the intraocular pressure constant gives a measure of the facility of the eye. The computation of facility from the increase in value of the force during the course of the measurement requires the use of the Imbert-Fick law. The application of the Imbert-Fick law for this purpose has been challenged (Ref. 1). The question raised is important in the evaluation of constant-pressure tonography and is examined in the experiments to be described in this report.

SUMMARY AND CONCLUSIONS

The Imbert-Fick law is assumed in constant-pressure tonography for computing the value of aqueous facility from the tonogram obtained in the measurement. A published result indicated that a large deviation from the theoretical Imbert-Fick law occurs in its application to tonography, a deviation that may require a substantial correction to the calibration charts used in this clinical procedure. Since we are generating the charts, it is important to know if such corrections are necessary.

Our results show that, in this application, deviations from the Imbert-Fick law are small compared with other errors, and special corrections for the deviations cannot be justified. Any corrections for the nonideal use of this law should await a new formulation of the law based on a theoretical treatment that includes the additional factors that can cause errors such as the finite thickness of the cornea, the bending forces of the cornea, and the surface tension of the tear film of the eye.

DISCUSSION

The Imbert-Fick law expresses the relationship of the area of flattening, A, of a thin spherical membrane by a flat surface as a function of the force, W, exerted by the surface against the membrane, and the pressure, P, on the inner side of the membrane with respect to the pressure outside:

$$A = 73.5 \frac{W}{P} ,$$

where A is in square millimeters, W is in grams, and P is in millimeters of mercury. In previous work (Ref. 1), the empirical formula for this relationship is given as follows:

$$A = 63.1 \frac{W}{P} + 0.67 .$$

The large deviation from the Imbert-Fick law is attributed to corneal bending forces, surface tension, and the finite thickness of the cornea. Since the use of the empirical formula gives a facility that is 27% lower than that of the Imbert-Fick law, a verification of the empirical relationship is indicated.

We have tested this application of the Imbert-Fick law with the apparatus shown in Fig. 1. Cornea from enucleated eyes were excised along a circle to include 1 mm of sclera beyond the limbus. The corneal specimen was then mounted in a plastic chamber that held the cornea in place by clamping onto the sclera. The chamber was then pressurized with fluid, and the cornea assumed its natural shape. Tonography could then be performed on the cornea. These experiments used eight rabbit corneas and two human corneas that were rejected as unsuitable donor material for keratoplasty.

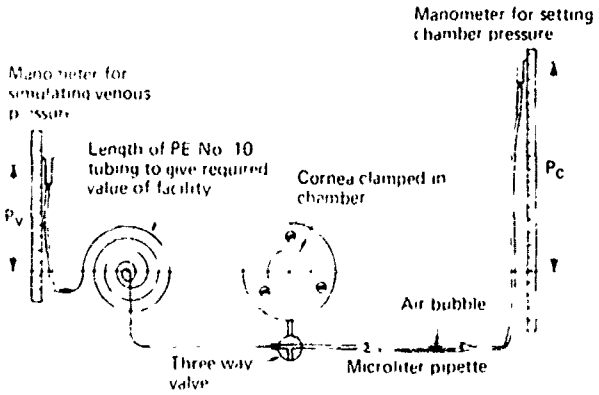

Fig 1 Apparatus for Testing Tonographic Instruments An excised cornea is clamped in a hollow fluid filled chamber so that the cornea will assume its natural shape for tonographic tests. A three-way valve allows fluid connections to either manometer and, in the position shown, allows a direct measurement of the facility (inverse of the resistivity) of the small diameter PE tubing (76 3/39)

For the test of the Imbert-Fick law, the three-way valve shown in Fig. 1 was turned to connect only the right-hand manometer. In the experiment, the flat surface of the applanator was applied with known values of force against the cornea clamped in the chamber. From the volume displaced (as measured by the bubble movement in the pipette) the area of flattening by the applanator was computed and plotted against the ratio of applanation force and chamber pressure. The results are shown in Fig. 2 for 50 trials at various values of pressure and force. The equation obtained by a least-square fit is:

$$A = 68.2 \frac{W}{P} + 3.5 \quad .$$

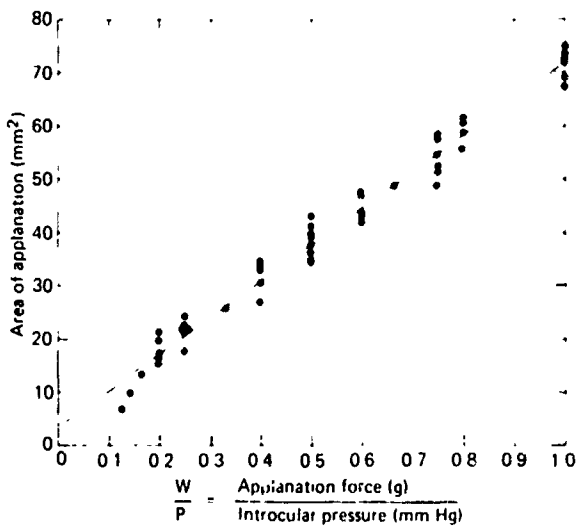

Fig. 2 The Area of Applanation (flattening by a flat surface) as a Function of Force Producing the Corneal Flattening and the Pressure Behind the Cornea Produced by the Column of Water in the Manometer for Setting Chamber Pressure. The straight line is a least-square fit to the 50 data points. (76-3/40)

Using this equation gives facility values averaging 6% lower than the Imbert-Fick law.

The same apparatus and the excised cornea were used to test the constant-pressure tonography procedure and to secure additional information about the validity of the Imbert-Fick law for this application. With the valve in the position shown in Fig. 1, the facility (reciprocal of the resistivity) of various lengths of tubing was measured. Then, by turning the valve to connect only the left-hand manometer, constant-pressure tonography was performed on the mounted cornea to determine the tubing facility with the tonography instrument. Figure 3 shows the result of measuring tubing facility by means of the tonography instrument, C_M, versus measuring the tubing facility by means of the air bubble movement in the graduated pipette, C_A. For each cornea, these measurements were performed using several levels of constant pressure. Since the results were uncorrelated ($p < 0.01$) with pressure, the data

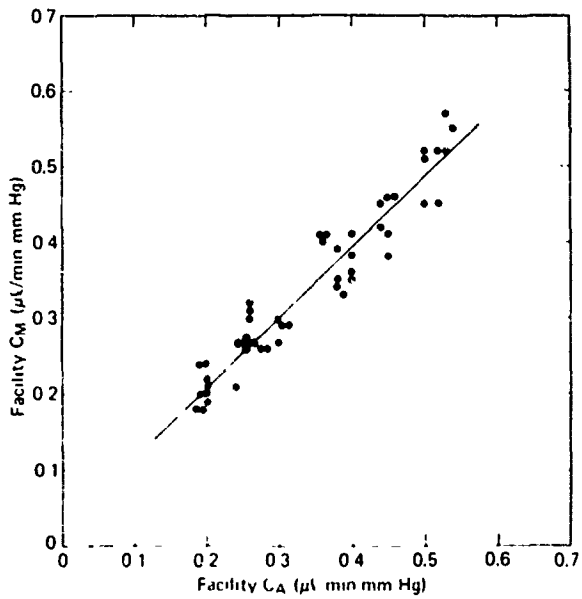

Fig 3 A Comparison of the Facility of Various Lengths of PE Tubing as Measured Directly by Bubble Movement in the Calibrated Pipette (C_A) and as Measured by the Constant-Pressure Tonographic Instrument (C_M) (76-3/41)

consisting of 50 trials were pooled, and the data points of C_M and C_A were fitted by least squares with the following results:

$$C_M = 0.94\ C_A + 0.02 \quad .$$

The regression line shows a close correspondence between the two methods of measurement. Using this equation to calculate C_M from C_A, the results yield answers with an accuracy of ±8% based on the standard error of the slope and intercept of the fitted line. The result is consistent with the data shown in Fig. 2, but not with the data published in Ref. 1. Therefore, based on our results, error compensation appears unnecessary in constant-pressure tonography for deviations from the Imbert-Fick law. A correction of 6% would contribute little to the accuracy because errors of about 20% from other causes already exist in this tonography procedure. Moreover, the correction that might be proposed for the average eye by using our empirical equation for the Imbert-Fick law has a magnitude not much different from the empirical equation's inherent error. Therefore, lacking better measurement precision, the correction to the law should come from a theoretical treatment of the Imbert-Fick law for this specific application.

REFERENCE

1. J. Gloster and E. S. Perkins, "The Validity of the Imbert-Fick Law as Applied to Applanation Tonometry," Exp. Eye Res., Vol. 2, 1963, pp. 274-283.

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING §39

Research by: FSE

Support: Collaborative Agreement with Rutgers University under USPHS Grant 5 P07 RR00643

L. J. Viernstein (APL) and I. P. Pollack (JHMI)

October 1975-September 1976

ARTIFICIAL INTELLIGENCE IN MEDICINE

A problem in clinical medicine now receiving increased attention is the growing gap between the large increase in medical knowledge and the limited information available to practicing physicians. The development of computer-based consultation programs is one approach to closing the gap. The computer programs contain a large data base of the latest medical information and use artificial intelligence techniques for securing the specific information required by the physician.

The Division of Research Resources of the National Institutes of Health (NIH) is supporting advanced methods of computer science for improving health care delivery. This division is supporting the development of computerized consultation programs in such areas as internal medicine, bacteriology, and ophthalmology.

We are aiding in the evaluation of a glaucoma consultation program developed at Rutgers University under the support of NIH. This is the first consultation program in which a number of medical centers throughout the country are linked to a common medical data base. The participants in the collaborative network are the Mt. Sinai School of Medicine, the Johns Hopkins Hospital, Washington University, the University of Illinois at Chicago, and the University of Miami. Each center has computer terminals connected to the TYMNET communication center via telephone. All the collaborators have access to the Sumex PDP-10 computer at Stanford University that provides a nationally shared resource for artificial intelligence in medicine. It is envisioned that local physicians ultimately will rent such terminals for securing consultation service and for obtaining the latest information in specific areas of medicine.

DISCUSSION

The objective of computer-based medical consultation programs is to provide physicians, especially in remote areas, access to the best thinking and knowledge available on various diseases, particularly those difficult to diagnose. The method is superior to a textbook search because initially the physician may not have a good idea of where the essential information exists in the literature. Aided by computer prompting, the physician enters information about the patient's condition. The computer does the search and analysis, and recommends additional tests if the information is not complete enough. If sufficient information is on hand, the computer returns to the physician a diagnosis and recommended treatment. The physician can demand and receive an explanation justifying the diagnosis and treatment and can be referred to relevant literature. Thus, by using the terminal, the physician is, in a sense, consulting with the group of medical specialists who have provided the information for the development of the consultation program and its data base.

The glaucoma consultation program in its present form leads the physician by displaying questions concerning the patient's condition. Most questions are arranged so that the physician can respond yes or no. Other questions require a numerical value from a test such as visual acuity, tonometry, aqueous outflow facility, or gonioscopy angle. The questioning becomes detailed only if the computer program develops evidence of pathology from the answers to the questions. A printout is generated in which the findings are reviewed together with their relationship with the past history of the patient (if such information was entered previously). Then the recommended diagnosis and treatment are given. On request, a justification for the conclusions is printed together with a brief explanation from current clinical and research papers on the condition diagnosed.

The general method (Ref. 1) used in the computer-based glaucoma consultation program is called CASNET (the causal associational network). An overview of the CASNET consultation program is shown in Fig. 1. The data acquisition module is controlled by the main command module, which selects the strategy to be used for securing the data. Once a lead is picked up, the structure of the questions follows a model of the suspected disease. The interpretation module applies the built-in rules for evaluating evidence, which consist of confirming or denying nodes in the causal network. The causal network rules propagate the evidence throughout the network and cause new states to be activated, triggering appropriate questions through the data acquisition module. When sufficient data have been gathered on the case, the weights set by the findings are summed along the various pathways. From this, a hypothesis is generated based on the classification rules in the program. After the diagnosis is given, the reasoning that led to it is printed out, giving (a) the causal pathways used to support the diagnosis, (b) direct evidence supporting the activation of states within the relevant pathways, and (c) the manner in which conflicts of evidence were decided. The diagnostic interpretation is used as the basis for therapy selection, making use of the programmed therapy rules appropriate for the diagnosis while taking into account characteristics of the individual patient. The motivation for the intensive modeling and development of the CASNET system derives from the prevalence and seriousness of glaucoma, a disease that, untreated, leads eventually to permanent blindness.

We have been contributing to the development of the glaucoma consultation program by entering patient information into the consultation program and comparing the computer responses with those of skilled physicians at the Wilmer Eye Clinic of Johns Hopkins. We have sent to Rutgers University over the same telephone network corrections and suggestions for improving the program. Conflicts do arise between the centers, especially in the area of treatment, and are usually resolved at general meetings of all the participants at Rutgers University. When conflicts cannot be resolved, the alternate treatments with their supporting

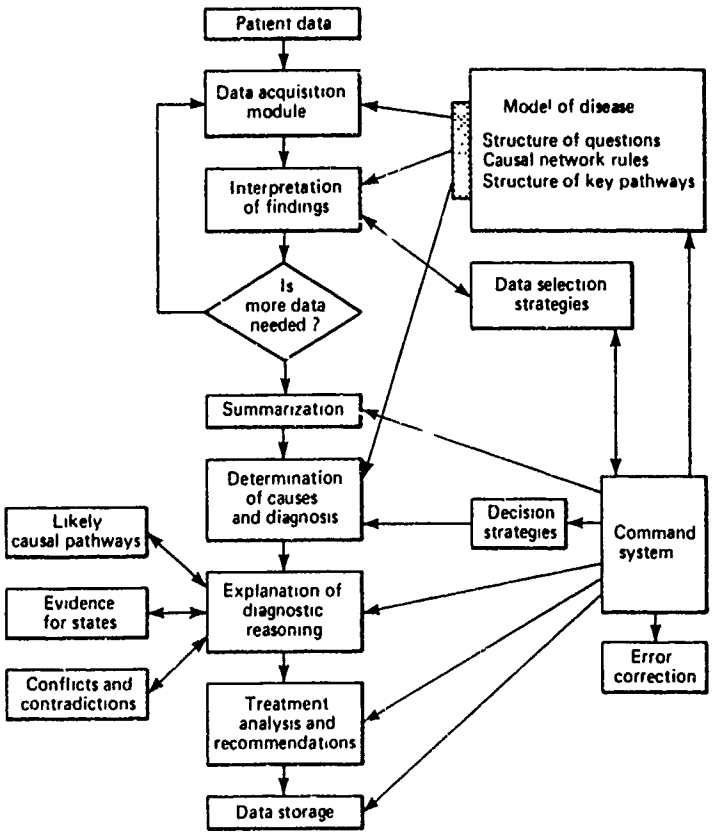

Fig. 1 Overview of the Organization of the CASNET Computer Program that will Provide Glaucoma Consultation Services to Physicians Anywhere in the U.S (76-3/43)

arguments are presented in the treatment section of the consultation program. The long-term storage of data will eventually build up evidence of the efficacy of different treatments.

FUTURE PLANS

Changes in the CASNET program have been numerous as a result of the contributions of glaucoma specialists at the various medical centers. The existing program is written in Snobol and Fortran, computer languages not suitable for frequent changes. Alterations of one part of the consultation program have led to difficulties in other parts. A radically revised consultation program written in Lisp, a list processing language, will soon be put on line. The Lisp language is more flexible and will allow independent changes to be made in the glaucoma consultation program without disrupting other parts of it.

We are also setting up a plan for pooling patient information from various parts of the country. For some diseases (for example, unilateral ocular hypertension), no single center has enough patients for a comprehensive study. The pooling of information from all the medical centers will allow the collection of data on a significant number of cases so that meaningful conclusions about the progress and treatment of patient populations may be made available to all ophthalmologists.

REFERENCE

1. C. A. Kulikowski, S. Weiss, and A. Safir, "Glaucoma Diagnosis and Therapy by Computer," Proceedings of Annual Meeting, Association for Research in Vision and Ophthalmology, May 1973.

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING §40
Research by: REM
Support: NIH Contract N01 EY-3-2139 (NEI)
B. F Hochheimer (APL) and A. Patz (JHMI)
October 1975-September 1976

INDICATOR DYE STUDIES

The morphological abnormalities of various disease states of the retina, choroid, and vitreous have been previously investigated and documented. However, the interrelationship between blood flow, vascular characteristics, and tissue metabolic requirements in the vasculature of normal and diseased eyes has not been adequately quantified.

Angiography is a noninvasive technique that can be applied to human subjects. Although fluorescein angiography has contributed significantly to the understanding of several retinal and choroidal diseases, there are still major limitations to their study, particularly to studying the choroidal circulation. For example, at the wavelength where sodium fluorescein emits energy, there is very low transmission through the pigment epithelium and xanthophyll pigment of the macula.

To overcome such obstacles, we are performing a systematic and comparative evaluation of indicator substances, with sodium fluorescein as a reference, in the study of retinal and choroidal circulations. Specifically, we are

1. Evaluating a list of dyes given by NIH,
2. Suggesting additional indicator substances,
3. Obtaining in vitro spectral characteristics of the indicator substances,
4. Testing the dyes for toxicity,
5. Performing animal angiography with fluorescent nontoxic dyes,
6. Evaluating the vascular morphology from the angiographic photographs, and
7. Making quantitative measurements of retinal and choroidal blood flow.

SUMMARY AND DISCUSSION

Nearly 700 dyes were screened during the period of the contract. The following water-soluble and nontoxic fluorescent dyes were found to be suitable for angiography:

Eosin Y
Erythrosin B
Phloxene B
Resazurin
Rhodamine S
Brilliant sulfoflavin
8-Hydroxy-1,3,6-pyrene trisulfonic and trisodium salt
Phloxine rhodamine
4,5-Dichlorofluorescein
NK-1460, NK-1469: These are catalog numbers of cyanine dyes produced by the Nippon Kanko Shikiso Kenkyusho Company of Japan.

One suitable absorption dye was found, Patent Blue.

Angiograms of rhesus monkeys have been made with all these dyes. In addition to the dyes that we have tested for fluorescence, solubility, and toxicity, several other untested dyes may be useful with respect to toxicity:

NK-1839
NK-1893
NK-2075
NK-2335
Rhodamine WT
Carbazine 720

FUTURE PLANS

This contract ended on 30 June 1976. An NIH-NEI grant proposal will be submitted 1 November 1976 for continuation testing of a selected set of these dyes for chronic toxicity and carcinogenic effects, and to improve the quality of the angiograms.

Nine papers written on the work performed under this contract are being submitted to technical journals for publication.

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING §41
Research by: CBP
Support: USPHS Grant EY-01008 (NEI)
R. W. Flower (APL) and A. Patz (JHMI)
October 1975-September 1976

STUDIES OF RETINAL ISCHEMIA

The objectives of this project are: (a) to investigate the separate contributions of the choroidal and retinal circulations to retinal tissue oxygenation; (b) to map the surface retinal oxygen tension (pO_2), determining areas of differences, if any, with special reference to the temporal periphery of the retina, the site of predilection for sickle cell and several related retinopathies; (c) to measure the arterio-venous difference of oxygen in different zones of the retina as an index of metabolic activity; and (d) to investigate further the effects of hyperbaric oxygen on retinal ischemia.

SUMMARY AND CONCLUSIONS

As indicated in the previous Annual Report (Ref. 1, procurement of microelectrodes having suitable characteristics for pulsed polarographic oxygen measurements has been a major problem. Since commercially produced microelectrodes proved to be highly unreliable and expensive, we endeavored to fabricate our own. The technique for production of beveled, glass-insulated, exposed platinum microelectrodes such as the sample shown in the scanning electron micrograph (Fig. 1) has been perfected in our laboratory. By greatly modifying the K. T. Brown beveling method for production of glass micropipettes, we are now able to produce quantities of these microelectrodes routinely, of which more than 90% meet the criteria for use in our experiments. These criteria are reiterated:

1. The electrodes must have small, sharp, and exposed noble metal tips (i.e., not covered by a membrane and not recessed) since the electrodes must be capable of sensing local electroretinographic potentials in addition to tissue pO_2 levels;
2. Since O_2 microelectrode measurements are affected by oxygen levels within approximately six (exposed metal) tip diameters, a requirement for diameters of less than 10 μm is imposed by the necessity to achieve a degree of spatial resolution while measuring pO_2 levels within the various tissue layers; and
3. The microelectrode tips must be sharp and have a fairly high degree of structural integrity in order to penetrate the intraocular tissues easily.

It was demonstrated that the minimum microelectrode impedance needed to interface with the polarographic electronics is approximately 16 MΩ (measured at 1 kHz). Inspection by scanning electron microscope of electrode tips ground to produce that particular impedance indicated that the average exposed platinum area is 50 μm². Thus, the microelectrodes have equivalent (exposed metal) tip diameters

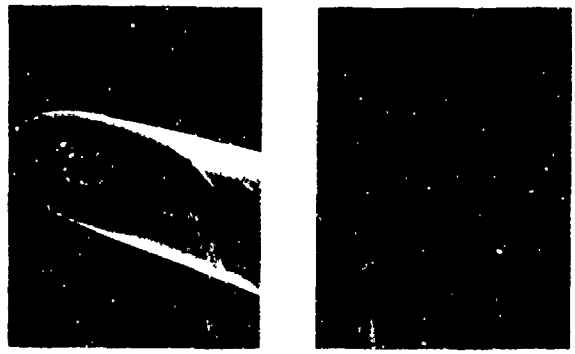

Fig. 1 Sample of Beveled, Glass-Insulated, Exposed Platinum Microelectrode. (76-3/52)

of 8 μm, which is well under the specified limit (criterion 2 above).

The bevel on the electrodes is usually about 30° (see Fig. 1). This produces a fairly sharp glass cutting edge that easily penetrates tissue, producing minimum trauma and minimum tissue drag (criterion 3 above). Nevertheless, experience indicates that for in vivo use the electrodes should be advanced to their farthest limits in the tissue and the measurements made as the electrode is withdrawn in order to avoid puckering the tissue.

Local electroretinographic potentials have been satisfactorily recorded with the microelectrode, and they produce linear pO_2 versus current calibration curves (criterion 1 above). These calibrations have been found to be stable over a period of several days in vitro, especially when the polarizing potential of -0.6 V DC is applied in single pulses of 400-ms duration. (Only one such pulse is required for each pO_2 measurement.)

Measurements of vitreal pO_2 levels were made in primate eyes while the animal was breathing first room air, then 100% O_2, and finally, a mixture of room air and humidified nitrogen. In general, changes in pO_2 resulting from breathing the different gas mixtures were more pronounced when measurements were made close to the retina than at a distance from it. Qualitative measurements were repeatable, but quantitative measurements were inhibited by the presence of an additional, unwanted current path between the platinum microelectrode and the Ag/AgCl reference electrode, apparently established in vivo between the platinum electrode and ground through the animal. Once the unwanted current pathway was located, an appropriate modification to the polarographic electronics was designed and implemented to nullify it. Measurements will be continued with the modified electronics.

FUTURE PLANS

During the final year of this study, the following specific objectives will be pursued:

1. To complete measurements of vitreal pO_2 levels in the eyes of primates while they breathe gases of varying O_2 content and then to extend the measurements to intraretinal and choroidal tissues;
2. By placing the O_2 microelectrodes in electrically conductive gels whose gas diffusion coefficients have been determined,

to attempt to demonstrate the presumed dependence of initial polarographic current decay on the O_2 diffusion characteristics of the medium; and

3. Upon successful completion of 2 above, to attempt to determine oxygen diffusion characteristics of intraocular tissues by analysis of current decay data recorded during collection of data for goal 1 above.

REFERENCE

1. Section 64, Annual Report, October 1974-September 1975, APL/JHU MQR/75-3.

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING §42

Research by: CBP

Support: Grant from Hynson, Westcott and Dunning, Inc. and USPHS Grant EY-01008 (NEI)

R. W. Flower

October 1975-September 1976

SIMULTANEOUS RETINAL AND CHOROIDAL ANGIOGRAPHY

This research program is an outgrowth of the Studies of Retinal Ischemia Program. As part of that program, it was necessary to develop a way to view routinely the flow of blood through the choroidal circulation. Having devised a method to do this simultaneously with standard fluorescein angiography, an effort will be made to evaluate the method in terms of its clinical usefulness.

SUMMARY AND CONCLUSIONS

As indicated in the previous Annual Report (Ref. 1), a standard Zeiss fundus camera has been modified by installing a continuous light source and a motorized 35-mm camera (capable of operating at 4 frames per second) to record angiographically the rapid sequence of choroidal dye-filling events. The modification consists of simple adaptors that can be easily applied to or removed from the fundus camera. Simplicity in design of the camera modifications was predicated on the idea that the technique of choroidal angiography must be refined to the point where it can be economically and reliably duplicated by other investigators so that the ultimate value of choroidal angiography can be determined. Although the adaptors were constructed for use on a conventional Zeiss fundus camera, similar adaptors can perform identical functions on other types of fundus cameras. The modifications are shown in Fig. 1 and are briefly described below.

An adaptor, containing a 132-mm field lens and a 55-mm f/3.5 relay lens as well as the indocyanine green (ICG) barrier filter, reduces the image size of the fundus to a diameter of approximately 17 mm in the photographic film plane. Reduction of the image size results in the availability of a higher luminous flux for the high-speed infrared film, assuring adequate exposure. A motorized Nikon 35-mm camera powered by a battery supply is attached to the adaptor. The fundus camera's usual xenon flash lamp is replaced by a General Electric FCS, 150-W quartz halogen lamp powered by 24 VAC supply. The small relay box synchronizes the usual Zeiss power supply with the 24 VAC supply.

Figure 2 shows a sequence of angiograms made on a human subject at a rate of 5 frames per second. (The pictures were made with a 35-mm motion picture camera having a 1/15-s shutter speed instead of the motorized 35-mm Nikon camera shown in Fig. 1.) The sequence of angiograms was made near the equator of the eye where the filling of a vortex venous system is demonstrated.

A method has also been perfected to quantify the fluorescence intensity of ICG and the fluorescence intensity or absorption of energy by any other dye (such as sodium fluorescein) simultaneously injected and photographed with ICG. From this data, dye concentration in individual ocular blood vessels may be determined. To the best of our knowledge, this technique is the first reported way to obtain these kinds of data from the eye noninvasively in a manner in which they can be truly quantified (Ref. 2).

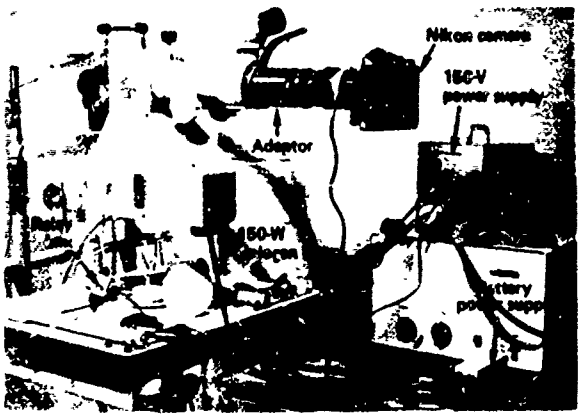

Fig. 1 Modified Standard Zeiss Fundus Camera. (76-3/53)

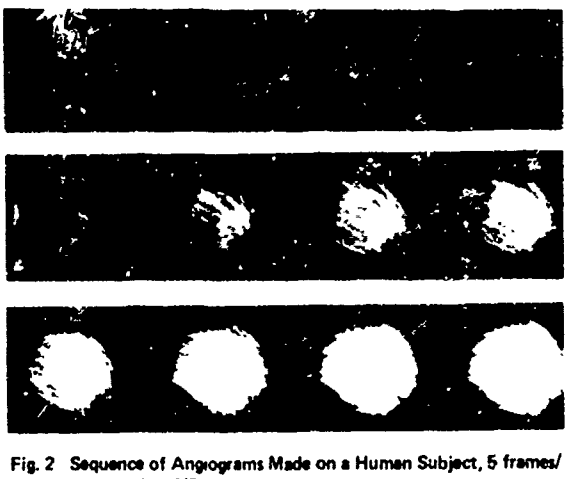

Fig. 2 Sequence of Angiograms Made on a Human Subject, 5 frames/second. (76-3/54)

Another area investigated during the past year was the effect of occlusion of choroidal arteries on the dynamics of choroidal blood flow. Although occlusions have been attempted in primate eyes with varying degrees of success, we were fortunate to be able to examine extensively one human case of central retinal artery occlusion in which segments of the choroidal circulation were also involved. The primate investigations are still going on, but the results of the human case study have been successfully used to explain the sequela of electroretinographic events and visual field anomalies observed in the patient (Ref. 3).

FUTURE PLANS

Optical components of the modified fundus camera have apparently been refined to the ex-

tent that resolution of choroidal vascular structures is limited by properties inherent in the eye itself. The modifications described in this report make it possible to achieve adequate temporal resolution in choroidal angiograms by using a continuous light source. Therefore, a larger group of investigators might now consider using the technique of choroidal angiography.

Future work to determine the ultimate clinical value of choroidal angiography must be performed by clinicians who routinely evaluate the intraocular circulations. Since several clinical investigators in the U.S. and Europe are currently using the technique and are obtaining technically satisfactory results, its clinical usefulness should be fairly well established within the next five years. During that time, we will attempt to render whatever technical assistance we can to our collaborators and will attempt to maintain active communication among them concerning their individual progress.

REFERENCES

1. Section 65, Annual Report, October 1974-September 1975, APL/JHU MQR/75-3.
2. R. W. Flower and B. F. Hochheimer, "Quantification of Fluorescent Dye Concentration in Vessels of the Ocular Fundus" (submitted to Vis. Res.).
3. R. W. Flower, P. Speros, and K. R. Kenyon, "Electroretinographic Changes and Choroidal Defects in a Case of Central Retinal Artery Occlusion" (Am. J. Ophthal., in press).

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING §43

Research by: RTP, CBP

Support: USPHS Grants NS-07226 (NINCDS) and EY-01008 (NEI), and APL

J. F. Bird, R. W. Flower, and G. H. Mowbray

October 1975-September 1976

ANALYSIS OF RETINAL RESPONSES

Electroretinographic (ERG) transient responses were elicited from monkey retinas by abruptly changing the periodicity of a rapidly intermittent (suprafusion) luminance, the same stimulus previously studied behaviorally in humans (Ref. 1). The suprafusion ERG transients were recorded simultaneously with visually evoked potentials (VEP) in the monkey cortex (Ref. 2) which we have analyzed and related to the human perceptions in earlier reports (Refs. 3 and 4). The ERG records have now been analyzed and interpreted by means of a theory of dynamic visual responses (Refs. 1 and 5). Preliminary results were indicated in Ref. 2, and complete results are detailed in a paper in preparation (Ref. 6). The ERG findings are the subject of the present report.

SUMMARY AND CONCLUSIONS

The suprafusion ERG transients elicited for various jumps in period ($t_1 \rightarrow t_2$) of the luminance modulation were recorded from one monkey in one experimental session (I) and from another in two other sessions (IIA and IIB). Standard ERG electrodes were used, and the transient was detected by averaging ≥ 50 responses at each period-jump tested in each session. Various pairs of periods ranging between 1 and 10 ms were used, such that different period-pairs (t_1, t_2) gave the same period-jump ($t_2 - t_1$). This tested the theoretical prediction that the suprafusion transient as a function of periods varies only as $t_2 - t_1$. The averaged ERG values were reduced to principal components (PC) (cf Refs. 3 and 4). The results of the PC data analysis verified the theoretical expectation, as shown in Fig. 1. Thus, the suprafusion ERG transient has the character of an elemental retinal response that is directly interpretable in a dynamic theory of vision (Ref. 5).

Theoretical analysis of the suprafusion ERG transient in terms of retinal dynamics reduces the data waveforms to two elemental components (Table 1). One that accounts for approximately two-thirds of the total ERG data variance is strictly linear (I) and is closely related to the simultaneous cortical VEP correlated previously with suprafusion perceptions in man (Refs. 3 and 4). The other component, which comprises approximately one-third of the ERG, is a rectification (R) that we find closely associated with amacrine cell activity in the retina (Ref. 6). Theory further indicates that high-frequency nonlinear ERG flicker offers separate measures of activities proximal and distal to the amacrine neurons. Thus, theoretical analyses of suprafusion ERG experiments might accomplish a virtual dissection of the retina.

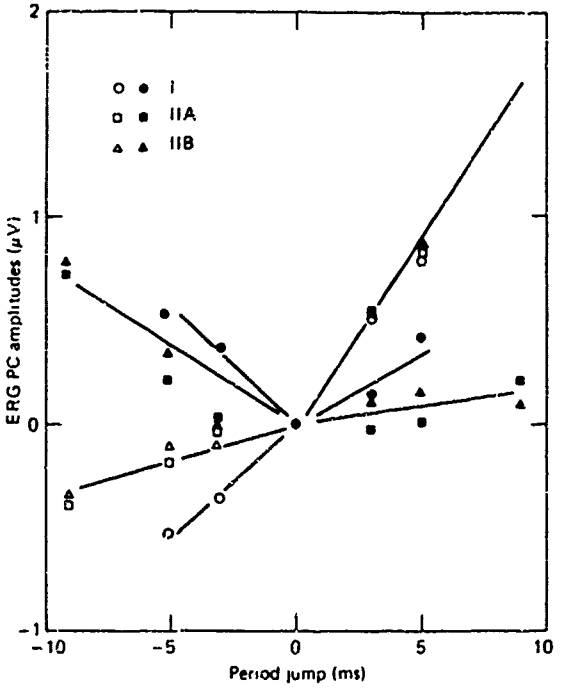

Fig. 1 Principal Components (PC) Amplitudes as Functions of Period-Jump (t_2-t_1). (Sessions I, IIA, and IIB; first PC open symbols, second PC solid.) (76-3/48)

Table 1
Elemental Components Variance (%)

Component	Session I (selected)	Session IIA (all)	Session IIB (filtered)
Linear	63	55	60
Rectified	31	29	36
Other	6	16	4

FUTURE PLANS

The theory has been broadened and applied to color vision dynamics, and a proposal for chromatic studies in collaboration with JHMI has been submitted to NIH. Related psychophysical, VEP, and ERG studies are under consideration.

DISCUSSION

The theory of suprafusion transients (Ref. 1) and an analysis of retinal dynamics (Ref. 6) showed that the ERG transient due to period-jump $t_1 \rightarrow t_2$ in a retinal illuminance modulated about a mean level I_g has the high-frequency approximation

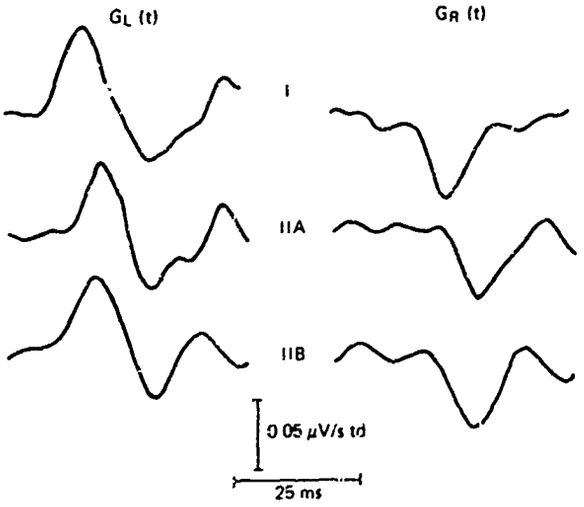

Fig. 2 Elemental Green's Functions for Linear (G_L) and Rectifying (G_R) Operation Determined in Sessions I, IIA, and IIB. (76-3/49)

$$ERG_{1\to 2}(t) \approx \tfrac{1}{4}I_s\cdot[(t_2 - t_1)G_L(t) + |t_2 - t_1|G_R(t)] \quad , \qquad (1)$$

where $G_L(t)$ and $G_R(t)$ are the elemental Green's functions for linear and rectifying retinal operations, respectively (cf Table 1). These elemental functions are determined from the measured ERG waveforms by a least-squares calculation. Figure 2 shows the calculated G_L and G_R from each of the three experimental sessions. The overall consistency among the three separate determinations appears satisfactory.

The linear element G_L bears a close relation to the cortical suprafusion VEP transients (Refs. 3 and 4) recorded simultaneously with the ERG, and its Fourier transform correlates well with neuronal and psychophysical sinusoidal responses, as indicated in a preliminary analysis (Ref. 5) and discussed further in Ref. 6.

The rectifying element G_R represents an essential nonlinearity of the retina, disclosed by the suprafusion technique to be unexpectedly substantial (Table 1). G_R possesses a number of features that implicate on-off amacrine cells as the origin, and possible sole agent, of the retinal rectification (Ref. 6). Briefly, the symmetric-rectification in the G_R term of Eq. (1) and the monophasic, single-peaked G_R form of Fig. 2 point to intracellular amacrine potentials as the source; the size, polarity, latency, and duration of G_R (Fig. 2) are only consistent with known extracellular and extraretinal signs of amacrine activity. On the hypothesis of an amacrine origin of G_R, it follows that harmonics generated by the retinal rectification in high-frequency ERG flicker offer a measure of separate retinal activities distal and proximal to the amacrine cells (Ref. 6). Thus, analysis of high-frequency ERG transients and flicker together might effect a noninvasive dissection of the retina.

REFERENCES

1. J. F. Bird and G. H. Mowbray, "Analysis of Transient Visual Sensations above the Flicker Fusion Frequency," Vision Research, Vol. 13, March 1973, pp. 673-687.
2. R. W. Flower, J. F. Bird, and G. H. Mowbray, "Retinal and Cortical Electrophysiological Responses to Instantaneous Frequency Shifts in Light Modulated above Fusion," Investigative Ophthalmology, Vol. 14, January 1975, pp. 75-78.
3. G. H. Mowbray, R. W. Flower, and J. F. Bird, "Visual Cortex Responses to Abrupt Changes in the Periodicity of Rapidly Intermittent Light," Electroenceph. Clinical Neurophysiology, Vol. 39, October 1975, pp. 305-312.
4. Section 66, Annual Report, October 1974-September 1975, APL/JHU MQR/75-3.
5. J. F. Bird, R. W. Flower, and G. H. Mowbray, "An Elemental Phenomenon of Vision — Suprafusion Transients: General Theory, Retinal-Cortical Manifestations, Potential Application," J. Theoretical Biology, Vol. 55, Part 2, December 1975, pp. 553-557.
6. J. F. Bird, R. W. Flower, and G. H. Mowbray, "Analysis of the Retina via Suprafusion Electroretinography," Vision Research (to be submitted).

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING §44
Research by: S2P
Support: NASA PR No. S-55719A
R. B. North, T. A. Fischell, and R. E. Fischell
June-September 1976

PAIN RELIEF BY ELECTRICAL STIMULATION

Among the more promising potential applications for the implantable, rechargeable, programmable stimulator that is evolving from the APL rechargeable cardiac pacemaker is stimulation of the nervous system to relieve chronic, intractable pain. Originally inspired by the now controversial "gate theory" (Ref. 1), electrical stimulation has been applied at many sites to produce analgesia (Ref. 2). The most widely known technique, implantation of stimulating electrodes over the spinal cord, has hitherto involved a major surgical procedure under general anesthesia (Ref. 3). Currently undergoing evaluation at the Johns Hopkins Hospital is a new technique whereby electrodes are inserted into the epidural space overlying the spinal cord via a Tuohy needle. Surgical risks are greatly reduced, and the use of local anesthesia permits the electrode's position to be optimized by feedback from the patient. This simple technique may have broad applicability to cases of intractable pain due to chronic low-back syndrome, terminal cancer, and many other disorders.

A follow-up study of patients with epidural stimulators was undertaken in the summer of 1976 to answer the following questions: (a) How effective is epidural stimulation for pain relief? (b) What, if any, are its side effects? (c) Is the externally powered stimulator currently in use adequate? (d) What is the patient's attitude toward a fully implanted system? (e) What are the design requirements for a fully implanted system?

SUMMARY AND CONCLUSIONS

Epidural stimulation, as judged by three subjective rating methods, successfully relieved chronic, intractable pain in from 23 to 26 of 31 patients studied. Reported improvements in the ability to perform various daily activities, and elimination of drug usage by many patients, corroborate this finding.

The side effects of stimulation, both as subjectively reported and as objectively measured by sensory testing, are not significant.

The externally powered, partially implanted system in current use suffers from problems of unreliability and inconvenience to the patient. Twenty-six of 31 patients reportedly would undergo a second operation solely to replace it with a totally implanted, rechargeable device. Experience with the present system dictates that such a device permit control of stimulation amplitude by the patient, with an on-off switching capability and perhaps automatic duty cycling.

DISCUSSION

Study Population. Dr. Donlin M. Long, Chairman of the Department of Neurosurgery at the Johns Hopkins Hospital, has so far implanted epidural stimulators in 37 patients. Of the 33 who could be reached for follow-up, two were eliminated from the study because of intercurrent use of other stimulation devices.

Demographic Data. The 31 patients studied (13 male, 18 female) have a mean age of 48. All are Caucasian; 27 are married; education and annual income are slightly above average. Fourteen are actively employed, three retired, and fourteen disabled.

Medical Histories. Twenty-four of the 31 patients studied suffer from chronic low-back syndrome, sometimes referred to as "failed back-surgery syndrome." One patient has cervical syndrome, or chronic neck pain; two are cancer patients; two are amputees — one with phantom limb pain, one with amputation neuroma; and one has muscular dystrophy. Collectively, these patients have undergone 161 operations, an average of 5.2 per patient, without satisfactory pain relief.

Study Methods. All 31 patients responded to a structured questionnaire: 17 were interviewed in person at APL, at Johns Hopkins Hospital, or at home, and 14 by telephone with the aid of written material sent through the mails. Since pain, like many symptoms of disease, is not manifest in any objective way, pain intensity and degree of relief were graded subjectively by each patient. Since stimulation of sufficient amplitude to provide pain relief produces a sensation by itself, a blind, controlled study was not planned, but some controls were generated inadvertently by device failures. Stimulation of the spinal cord does produce objective neurophysiological changes that correlate with pain relief (Ref. 4), but the ultimate criterion of success for this technique, as for more drastic measures undertaken in the past, is a patient's subjective impression. There exists no adequate animal model by which these methods might be evaluated.

Results. The efficacy of epidural stimulation for pain relief was quantified in three ways. First, each patient was asked what percentage of time he experienced each of five degrees of pain ranging from no pain to excruciating pain, both with and without stimulation. The resulting profile for the average of 31 patients is presented in Fig. 1. Second, each patient was asked to compare the relief provided by epidural stimulation with that resulting from previous operations. Eighteen rated stimulation as much more effective, five as slightly more effective, four as about the same, one as slightly less effective, and two as much less effective. (One patient had had no previous surgery.) Third, each patient was asked whether, in view of the hospitalization, expense, and discomfort associated with the procedure, he would go through it all again for the same degree of pain relief. Twenty-one answered emphatically yes, five yes, three no, one emphatically no, and one was neutral.

Efficacy was indirectly measured by asking each patient to rate the difficulty he encountered as the result of pain in performing various activities, with and without stimulation. Significant, and in many cases dramatic, im-

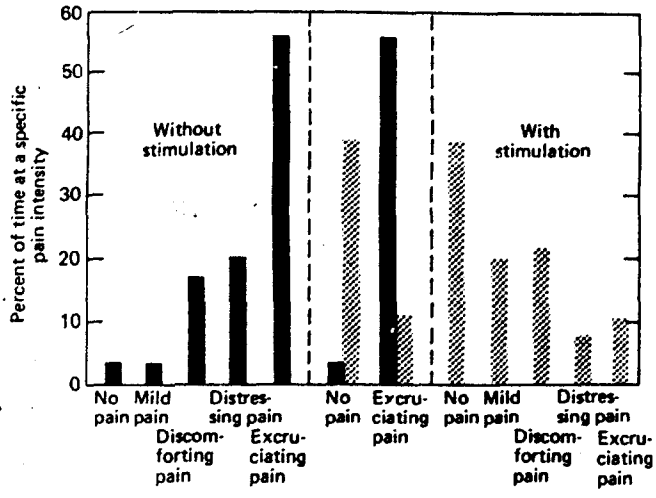

Fig. 1 Effect of Epidural Stimulation on Patient's Pain Experience. (76-3/34)

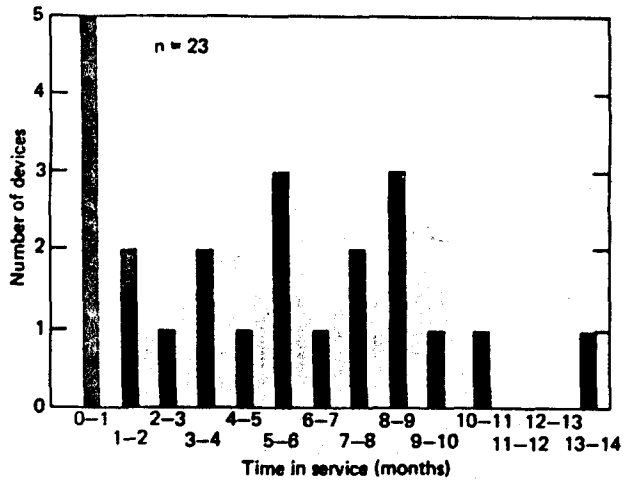

Fig. 2 Devices Still in Service (as of August 1976). (76-3/35)

provements were reported by most patients in their ability to sleep, sit up, stand, walk, eat at a table, dress, climb stairs, and have sexual relations. Although still subjective, this criterion of efficacy reflects a change in lifestyle more tangible than impressions of pain relief.

Epidural stimulation reportedly enabled most patients to decrease or eliminate the use of narcotic and nonnarcotic analgesics, sedatives, tranquilizers, and antidepressants; altogether, intake of 19 different drugs was affected. In particular, use of Percodan and Demerol, both strong narcotic analgesics, was curtailed by 15 and 7 patients, respectively.

The side effects reported by each patient, in response to specific questions, are summarized in Table 1. Undesirable side effects are relatively few; the two patients who reportedly strain to urinate during stimulation merely turn off their stimulators as necessary. One patient reports the opposite effect. Reported relief from constipation in eight patients is felt to be an indirect result of stimulation; curtailment of narcotic use is felt to be the direct cause.

To assess the potential risk that a patient might injure himself unknowingly because of stimulation-induced sensory loss, objective tests were devised to measure the function of the three major ascending sensory tracts in the spinal cord. Simple touch threshold, two-point discrimination, and temperature discrimination were tested on hand and foot, above and below the level of stimulation. No significant changes were produced by stimulation in the 10 patients studied.

At the close of the study, 23 of the 31 patients had functioning implants whose length of service ranged up to 13 months (Fig. 2). In view of reported decreases with time in efficacy of conventional dorsal column stimulators, it is of interest that eight of our patients reported increases in efficacy, and only one reported a decrease, over a 5 to 6 month median experience. Placebo effect, which can be a significant factor in pain studies, is unlikely to last this long. Spontaneous electrode displacements causing loss of reported analysis but not of stimulation sensation were further evidence that the reported pain relief was not a placebo effect.

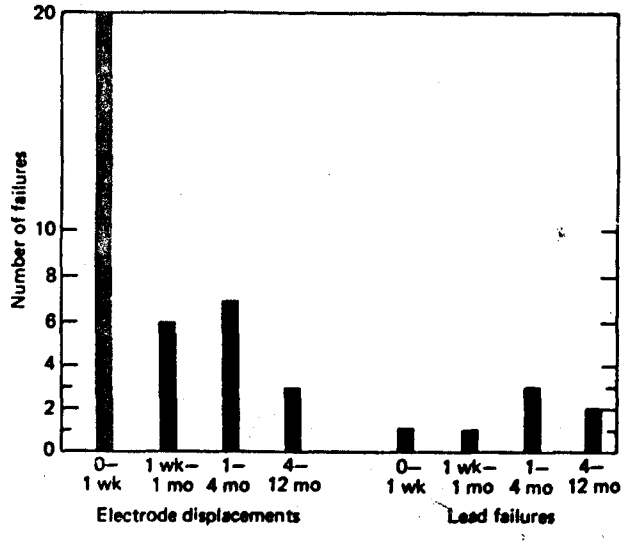

Fig. 3 Reliability Time to Failure by Mode. (76-3/36)

A number of malfunctions of the existing system were encountered. As shown in Fig. 3, 19 patients experienced a total of 36 spontaneous electrode displacements, requiring minor surgery for repositioning of the lead and electrode to the appropriate spinal level. Attachment of the present lead to the lumbar fascia with an encircling silk suture is apparently inadequate; use of an anchoring sleeve and adhesive is contemplated as a solution. Failures in seven patients of the lead itself are a related problem.

The implanted, passive receiver in the system is energized by an overlying antenna, taped to the skin and connected to an externally worn RF transmitter. Failures of this antenna were ubiquitous – in fact, two are supplied with each device. Eighteen patients report discomfort due

(continued)

Table 1
Side Effects Reported by Patients

Effect	Undesirable	Neutral	Desirable
Local sensation caused by stimulation	Unpleasant (1) Burning (1)	Neither pleasant nor unpleasant (17) Tingling (26) Pulsating (8) Vibrating (4) Buzzing (1)	Pleasant (13)
Sensory effects	Metallic taste in mouth (1) Slight numbness in hand (1) Slight numbness in one leg (1) Slight numbness below waist (1) Slower reaction to heat (1)	No change (25)	Better sensation in feet (1)
Motor effects	More difficult to walk (1) Slight decrease in strength (1)	No change (24)	Improved coordination (4) Slight increase in strength (1)
Changes in bladder habits	Must now strain to urinate (2)	Urinates more often (4) Urinates less often (1) No change (23)	No longer strains to urinate (1)
Changes in bowel habits		No change (22)	Relief of constipation (8) Relief of spastic colon (1)
Summary	11	159	29

to the external coil; three report constant and five occasional slippage of the coil resulting in loss of stimulation. Seventeen report that it is inconvenient to wear the external transmitter; for reasons of convenience and cosmesis, 10 occasionally endure pain rather than wear the device. Twenty-six of the 31 patients stated that they are willing to undergo a second operation solely to replace their present system with a fully implantable device, requiring weekly recharging.

A survey of patient usage of the existing device suggests that a totally implantable, rechargeable, remotely programmable device must be capable of delivering 4 to 10 V pulses, 0.3 to 1 ms in width, at a rate of 12 to 200 pulses/s. The attendant average current drain, ranging from 0.01 to 1 mA, dictates recharge intervals ranging from 4 to 25 days – an interval acceptable to the patients studied. While the physician alone will control most output parameters, the patient must be able to turn the device on and off and, since 21 of 31 patients report that amplitude changes are necessary to maintain pain relief during postural changes, it will be necessary to control amplitude remotely as well.

Finally, since pain relief may lag behind the onset of stimulation by from 1 to 120 min. and may persist for up to 12 h after stimulation ceases, automatic duty cycling of the device on and off may be desirable, to conserve power and to lessen the possibility of neuronal fatigue.

REFERENCES

1. R. Melzack and P. D. Wall, "Pain Mechanisms: A New Theory," Science Vol. 150, 1965, pp. 971-979.
2. D. M. Long and N. Hagfors, "Electrical Stimulation in the Nervous System: The Current Status of Electrical Stimulation of the Nervous System for Relief of Pain," Pain Vol. 1, 1975, pp. 109-123.
3. C. N. Shealy, J. T. Mortimer, and H. P. Reswick, "Electrical Inhibition of Pain by Stimulation of the Dorsal Columns," Anesth. Analg. Vol. 46, 1967, pp. 489-491.
4. R. D. G. Blair, R. G. Lee, and G. Vanderlinden, "Dorsal Column Stimulation: Its Effect on the Somatosensory Evoked Response," Arch. Neurol. Vol. 32, 1975, pp. 826-829.

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING §45
Research by: RTP, F3E, and REM
Support: USPHS Grant NS 07226 (NINCDS)
R. A. Meyer, S. F. Haase, and R. C. Benson (APL), and S. E. Poduslo and G. M. McKhann (JHMI)
October 1975-September 1976

AUTOMATIC CELL COUNTER/ SORTER

Flowing-cell sorting techniques are being investigated for separating specific brain cell types. Cell parameters that could be measured in a flowing-cell sorting system are being studied. Cell specific antisera that can be labeled with a fluorescent tag have been obtained for rat neurons and rat astrocytes. The light scattering patterns of different types of brain cells have been measured using a static optical system. A statistical analysis of these data indicates that neurons can be separated from neuronal debris based on light-scatter information. Neurons have been found to remain intact after flowing through a Becton-Dickinson flowing-cell sorter.

INTRODUCTION

The ability to isolate bulk samples of specific brain cell types is presently limited by the discrimination capability of sucrose gradient techniques. Isolation techniques based on other discrimination parameters are required to improve the isolation of specific cell types. Flowing-cell sorting systems have been used by others (Refs. 1 and 2) to separate single cells in suspension from other types of tissue. These systems inspect individual cells and identify them by measuring the fluorescence of specifically absorbed dyes, cell resistance (i.e., Coulter volume), and small-angle light scatter (i.e., less than 2°), which is used to estimate cell volume (Refs. 1 and 3). Also, large-angle light scattering can be used to provide information about the internal structure of the cells (Refs. 4 and 5).

DISCUSSION

Techniques have been developed for placing neurons and oligodendroglia that have been isolated by our sucrose gradient techniques (Ref. 6) into a standard tissue culture medium, and for maintaining the cells for a period of time. Cell viability has been tested by measuring the incorporation of precursor radioisotopic amino acid and uridine into trichloroacetic acid precipitable material (Ref. 7).

Work has continued on the development of cell specific antisera. When antineuron antiserum is absorbed with liver, red blood cells, and astrocytes, the antiserum still reacts with neurons (as indicated by a conventional fluorescent antibody assay), and thus the antiserum is specific to neurons. Antiserum specific to rat astrocytes has also been developed. Antiserum to oligodendroglia has been obtained by injecting rabbits subcutaneously. The specificity of this antiserum is presently being determined.

The light-scattering patterns of isolated brain cells have been measured using a sophisticated light detector consisting of 32 concentric ring detectors and 32 wedge detectors. Recently, data have been taken from preparations of rat neurons that also contained other cellular and subcellular particles. In order to perform a statistical analysis of the light-scattering data, two cell classifications were established. A "good cell" was a neuron that appeared intact when viewed using a phase-contrast microscope. (This identification is based on our previous experience with isolated cells.) A "bad cell" was everything not classified as a good cell. The bad cells included granular neurons, nuclei, subcellular debris, red blood cells, oligodendroglia, capillaries, and unidentifiable debris. Data were taken from 97 good cells and 106 bad cells.

The ability to discriminate between the good and bad cells has been estimated using a feature-reduction technique patterned after that discussed by Whitney (Ref. 8). An 11-nearest-neighbor classification rule has been used in a "leave one out" scheme to estimate the probability of correct classification. The decision rule is to decide "good" if at least 10 of the 11 nearest neighbors have been labeled "good" by a human observer, with nearness of neighbors defined by the Euclidian distance. Such a decision rule in a feature-reduction program provides a useful set of parameters at the small false-alarm rate required for high final purity if the cells were actually being sorted.

The best set of parameters for separating the two classes is shown in Fig. 1, where parameter values are plotted in the x and y directions with the number of cells shown by intensity. (Data from the detector elements have been combined in groups to reduce the number of measured features and thus satisfy Foley's criterion (Ref. 9) that the number of samples per class should be at least five times the number of parameters measured per sample.) Besides the composite histogram that includes all cells, histograms are also shown for each class to indicate the density overlap between classes. Because of the overlap between classes defined in this manner, the best sorting that can be achieved is about 30% detection with a false-alarm rate below 5%. However, most of the overlap between classes is due to the granular neurons in the bad cell class. If the decision were made to accept granular neurons in the good cell class, the separation between classes would be more complete. In order to determine the improvement, the cells have been relabeled, resulting in a data base of 140 good cells and 63 bad cells. The best set of parameters for separating the classes of all neurons is shown in Fig. 2. The feature-reduction routine indicates that about 70% of the neurons can be detected with a false-alarm rate below 5%. It thus appears that light-scatter information can be used to distinguish between neurons and neuronal debris.

Recently cultured brain cells have been run through a Becton-Dickinson flowing-cell sorter at NIH. After passing through the flow system, neurons remained intact and appeared morphologically the same as cells that did not flow through the system. There did not appear to be an increase in the granular neurons or neuronal debris in the sorted-cell population. A light-scatter histogram was obtained for the neuron prepara-

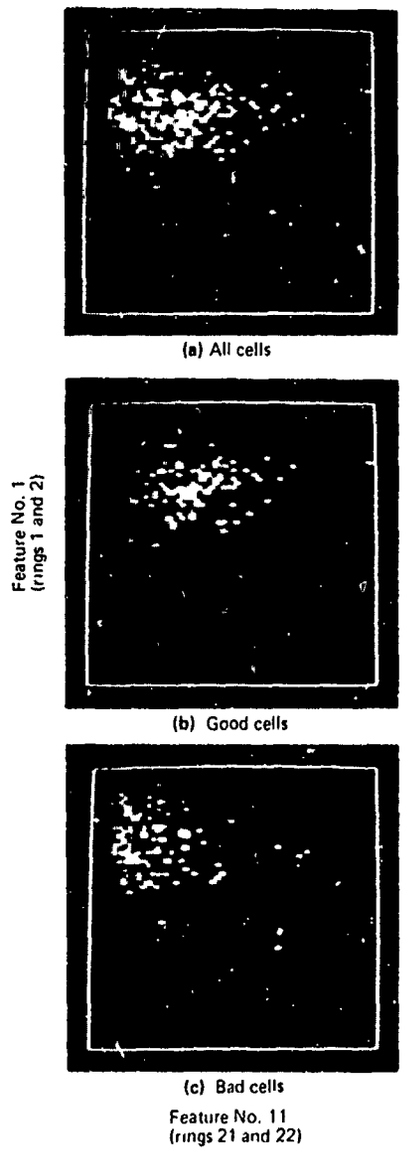

Fig. 1 Two parameter histograms of light scatter data for brain cells with number of cells shown in intensity: (a) histogram for all 203 cells in the study; (b) histogram for 97 intact neurons labeled as good cells; and (c) histogram for 106 objects in the bad cell classification which includes granular neurons, nuclei, subcellular debris, red blood cells, oligodendroglia, capillaries, and unidentifiable debris. (76-3/44)

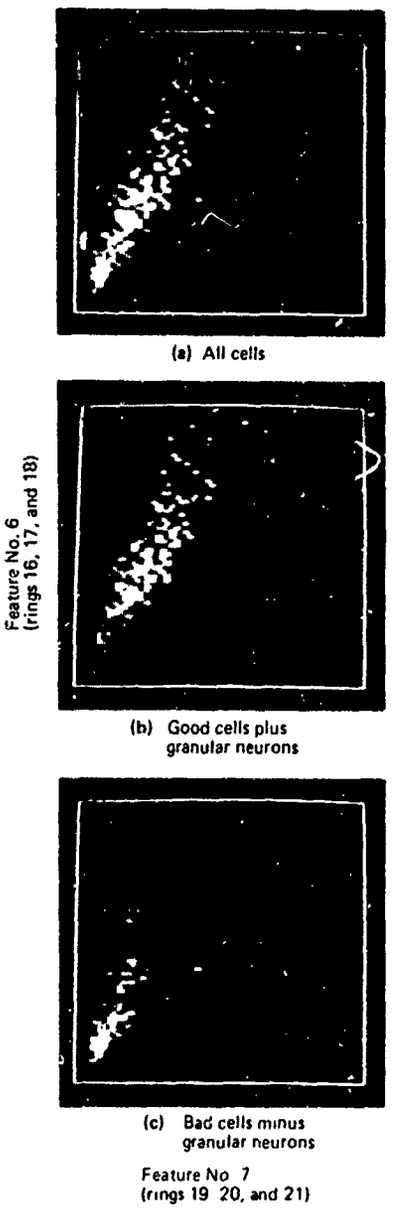

Fig. 2 Two-parameter histogram of light scatter data for (a) all cells in the study, (b) good cells plus granular neurons, and (c) bad cells minus granular neurons (76 3/45)

tion using the available light-scatter detector (a single detector covering the angular region of approximately 1 to 7°). The light-scatter histogram had a single peak and a long tail and appeared to be similar to a log-normal distribution. This distribution was divided approximately in half and cells that had a low light-scatter signal were separated from cells with a high one. The resulting cell population from the low light-scatter signal sort consisted of a larger percentage of granular neurons and neuronal debris than did the cell population from the high light-scatter signal sort. Therefore it appears that this simple low-angle light-scatter signal can be used to separate granular neurons from intact neurons. No clogging of the system was observed, even when samples were run through the system at flow rates as high as 4000 cells per second.

Fluorescence spectra were obtained of the media in which the cells are maintained (modified Eagle's medium (Dulbecco's) and phosphate-buffered saline (PBS) medium). An argon ion laser was used as the excitation source, and

(continued)

the fluorescence was detected at 90° from the excitation light with a scanning monochromator and photomultiplier tube. It was determined that several constituents (mainly riboflavin and phenyl red) of Dulbecco's medium fluoresce quite strongly. This rather strong fluorescence could be a problem in fluorescence-activated cell sorting where the cell fluorescence may be comparable to that of the medium. Weak fluorescence of PBS was detected, although the fluorescent molecule has not been identified. The intensity of PBS fluorescence is much lower than that of Dulbecco's medium; hopefully, it will not present a problem in the sorter. It was also determined that, using wideband spectral filters, the sensitivity of detecting fluorescence in aqueous media is limited by the Raman scattering from the water molecules. It may be possible to increase the sensitivity by using appropriate filters and excitation wavelengths to suppress the Raman scattering.

FUTURE PLANS

We will study the light-scattering patterns of a large number of cells to determine which light-scattering parameters are important for recognizing different brain cell types. We will also study different cell-specific fluorescent probes. Brain cells will be run through sorters at other research laboratories to determine how well they survive the transit through the sorter as well as to determine if parameters currently available on sorters can be used to separate different cell types. Fluorescent micrographs have indicated that neurons may autofluoresce. We will try to verify this by measuring the fluorescence spectra of bulk samples of specific cells. The fluorescence intensity could then be determined, the fluorescent species identified, and cell specificity determined.

REFERENCES

1. J. A. Steinkamp et al., "A New Multiparameter Separator for Microscopic Particles and Biological Cells," Rev. Sci. Instrum., Vol. 44, No. 9, 1973, pp. 1301-1310.
2. W. A. Boner et al., "Fluorescence Activated Cell Sorting," Rev. Sci. Instrum., Vol. 43, No. 3, 1972, pp. 404-409.
3. P. F. Mullaney and P. N. Dean, "The Small Angle Light Scattering of Biological Cells," Biophysical J., Vol. 10, No. 8, 1970, pp. 764-772.
4. A. Brunsting and P. F. Mullaney, "Light Scattering from Coated Spheres: Model for Biological Cells," Appl. Optics, Vol. 11, 1972, pp. 675-680.
5. R. A. Meyer and A. Brunsting, "Light Scattering from Nucleated Biological Cells," Biophysical J., Vol. 15, March 1975, pp. 191-203.
6. S. E. Poduslo and W. T. Norton, "Isolation of Specific Brain Cells," Methods in Enzymology (Colowick and Daplan, eds.), Academic Press, Vol. 35, 1975, pp. 561-579.
7. S. E. Poduslo, H. F. McFarland, C. D. Kroen, K. L. Miller, and G. M. McKhann, "Metabolic Properties of Isolated Brain Cells," Int. Soc. for Neurochem., Vol. 5, 1975.
8. A. W. Whitney, "A Direct Method of Nonparametric Measurement Selection," IEEE Trans. on Comp., September 1971, pp. 1100-1103.
9. D. H. Foley, "Considerations of Sample and Feature Size," IEEE Trans. Information Theory, Vol. IT-18, No. 5, September 1972, pp. 618-626.

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING §46
Research by: F1C
Support: USPHS Grant NS-07226 (NINCDS)
H. R. Biltner (APL) and J. C. Houk (JHMI)
October 1975-September 1976

MUSCLE PULLER

Research of neuromuscular control mechanisms requires a means of controlling muscle displacements precisely and of measuring the reflex forces of certain muscles accurately. The versatile muscle puller system, designed and developed at APL, provides one-dimensional control and measurement of muscle displacement, velocity, and force.

For 2 years, the muscle puller has been used to study stretch receptors and feedback characteristics of primary spindle receptors. The program has now been completed, and the muscle puller is being readied for a comprehensive study of the animals' reactions to load disturbances applied to the limb. In the new program, the muscle puller will be used to simulate physiological conditions required to study these reaction responses.

DISCUSSION

The muscle puller system offers accurate displacement control over a range of 0.01 to 10 cm. Linear rates of displacement (ramps used to elongate or relax muscle length) as large as 250 mm/s are readily generated with the system.

During the reporting period, no additional modifications to the system were required. The system (reported in Ref. 1) has been used extensively during the full 12-month period covered by this report.

FUTURE PLANS

The muscle puller is now being modified so that it can be manipulated by monkeys in a fashion that mimics normal physiological loading conditions of the upper and lower limbs. A new force-sensing unit is being installed, and other modifications are being made to permit the use of the muscle puller as a force transducer for these studies. We anticipate that the muscle puller will be used in this new application most of next year. In conjunction with the new program of studies, a newly developed mechanical stimulator (reported elsewhere in this Annual Report) is being set up to study the responses of human subjects to load perturbations. Much of the data collected in the two study programs will be compared to assess the similarity of reactions to prescribed load changes. Longer range plans will use central nervous system recordings of the animal to determine the relationships of neurophysiological mechanisms to muscle response.

REFERENCE

1. Section 69, Annual Report, October 1974-September 1975, APL/JHU MQR 75-3.

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING §47
Research by: F1C
Support: USPHS Grant NS-07226 (NINCDS)
H. R. Bittner (APL) and J. C. Houk (JHMI)
October 1975-September 1976

NEUROMUSCULAR EVALUATION SYSTEMS FOR HUMAN SUBJECTS AND ANIMALS

A new program of research studies for evaluating neuromuscular control of human subjects is being developed in a laboratory in the Department of Physiology at JHMI. New equipment, designed to control static and dynamic displacements and forces as applied to human limbs has been completed in prototype form. This new equipment for human neuromuscular studies and the muscle puller system (reported elsewhere in this Annual Report), used for animal studies, will be set up in a back-to-back laboratory arrangement at JHMI. Comparative studies and correlations of response data resulting from similar studies involving humans and monkeys will be possible when the two systems are completed.

DISCUSSION

The Neuromuscular Evaluation System for humans uses a precision electromechanical stimulator designed to provide accurate linear displacements and velocities along a single axis. The stimulator is operated as a force-error nulling servo using a commercial load cell as the force-feedback sensing element. A force-feedback loop is closed through a single integration network on a minor velocity feedback loop to achieve overall control that simulates inertial-type loading of the limbs. In this mode of operation, the stimulator is commanded to produce an "apparent mass" load change that is experienced by the subject. EMG (electromyograph) recordings and recordings of position, velocity, and force error are used in the analysis of the subject's muscle response.

The prototype stimulator unit can deliver high force levels (60 kg continuous and 230 kg peak load) and a moderate range of linear velocities (up to 0.25 m/s). Due to the wide range of load displacements and velocities needed to study many different points of the human limbs, we anticipate further development of additional similar stimulator units at a later date. An example would be a unit capable of moderate force levels, long travel (up to 1 m), and high-velocity movement (up to 2 m/s). Such a unit will be required for the evaluation of the response properties of unloaded limbs.

FUTURE PLANS

The prototype stimulator has been fabricated and bench-tested at APL and will soon be transferred to the neuromuscular laboratory in the JHMI Department of Physiology. The next phase of the development will be that of interfacing the stimulator with the PDP-11 computer in the laboratory. Extensive use of the system in a study involving normal volunteer human subjects is anticipated during the next year.

Plans and formal proposals have been submitted to HEW for funds to develop a new class of neuromuscular stimulator units. These units will provide a means of studying multidimensional motions and complex muscle responses of humans and animals. The design of this new class of stimulators will provide features for measuring three or more degrees of limb motion and active mechanisms for applying composit disturbance forces to impede the subject's motion in reaching out to an array of targets. The development efforts for these multi-axis stimulators are planned to begin in May of 1977.

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING §48
Research by: F1C
Support: USPHS Grant NS-07226 (NINCDS)
H. R. Bittner (APL) and S. W. Brusilow (JHMI)
October 1975-September 1976

SCALA MEDIA PRESSURE MEASUREMENTS

A pressure-measuring transducer has been developed to measure hydrostatic pressure and to monitor changes of pressure in the scala media compartment in the inner ear of living guinea pigs. The design of the transducer disallows appreciable changes in the internal volume of the enclosed scala media, thus preserving the biological integrity and function of the inner ear. The technique that has been developed and proven requires venting of the biological pressure through a glass-drawn micropipette used to penetrate the membrane wall. The system operates to null the capillary flow through the pipette tip by applying and controlling an opposing pneumatic pressure applied to the external side of a compliant interface separating the two pressure chambers. Measurement of the pneumatic pressure thus becomes a measure of the hydrostatic pressure in the scala media.

DISCUSSION

The pressure transducer has been used extensively during this reporting period to measure hydrostatic pressure in scala vestibuli and scala media, two compartments in the inner ear. Flow through the pipette tip has been nulled by means of a manual pneumatic pump to provide a balance offset pressure.

One major change in the transducer design was made during the reporting period. The stiff ground-plane diaphragm was replaced by a thinner, more compliant diaphragm to eliminate pressure measurement uncertainties attributed to microscopic motions of the stiff diaphragm. By replacing the stiff ground plane with a more compliant diaphragm, and by widening the separation between the parallel plates of the capacitance pickoff, we have effectively reduced the system's sensitivity to mechanical and thermal instabilities. Although sensitivity to changes in flow through the pipette tip was also reduced by making these design changes, the sensitivity was recaptured by adding electrical gain in the summing amplifier used to amplify the flow error signal. The changes have resulted in a marked improvement in the short-term stability of the output signal, thus making a better case for closing the flow-nulling servo loop.

A new transducer design is being investigated. It will incorporate the feature of monitoring endolymphatic potentials through the micropipette, allowing simultaneous measurements of pressure and electrical potential of the biological fluid in question. The measurement of endolymphatic potential is of extreme importance in determining when the pipette tip penetrates the small scala media compartment.

FUTURE PLANS

This project is funded through May 1977. A proposal has been prepared and submitted to extend and expand the project effort for one additional year. Fabrication of a new transducer that incorporates the feature of measuring endolymphatic potentials is planned in the coming months. Following that design change, we intend to close the pneumatic servo loop, thereby allowing the pressure measurements to be made by one investigator instead of the two now required.

The new grant proposal calls for the design of a microperfusion pump to be used in conjunction with the pressure transducer in making pressure/volume studies of the major compartments of the inner ear. Additional studies will correlate measured pressure with a buildup of volume induced by injecting certain pharmacological agents.

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING §49
Research by: REM
Support: USPHS Research Grant NS07226 (NINCDS)
R. E. Walker (APL) and J. C. Houk (JHMI)
October 1975-September 1976

CRYOPROBE FOR REVERSIBLE COOLING OF THE CORTEX

Experiments are in progress at the Johns Hopkins School of Medicine to study the effects of hypothermia on specific neural paths and to use the effects in studying other neural properties. An earlier project involved the cooling of the sciatic nerve of a rat or rabbit to study the effect of blockage of the nervous system on neuromuscular functions. Specific cryoprobes are still being provided for that study. A more recent need to provide cryoprobes for reversible cooling of the cortex is described here.

SUMMARY

In vivo experiments are conducted with trained monkeys using an implanted thermode designed to provide local reversible cooling or heating of selected areas of the cortex. The principle adopted in the design of this thermode is that of liquid cooling of a heat exchanger that is implanted over a selected area of the cortex. Two designs have been investigated by the members of the Department of Physiology. One of them appears to be adequate.

DISCUSSION

An analysis of a model of an end-cooled metal thermode immersed in living tissue indicated that (with appropriately selected materials) adequate cooling with acceptable thermal gradients should be achievable with a thermode of a simple design. This approach offers the significant advantage that the more costly heat exchanger assembly could be made detachable from the implanted thermode and, therefore, could be used in other implanted thermode experiments. Instrument development has followed this simple, straightforward concept.

The first design consisted of a simple copper thermode mounted in a thin stainless-steel cup. The thermode was gold-coated for chemical inertness and affixed to the skull with acrylic cement, with the copper thermode in contact with the cortex. To the protruding end of the copper thermode was attached a detachable heat exchanger consisting of a resistance heater and a coil through which cooled methyl alcohol was circulated. Limited tests made with this thermode system indicated that it did not have the desired dynamic range and rapid response characteristics. Further, as experiments proceeded, it became evident that larger area thermodes requiring even greater heat-exchanger capabilities would be needed. As a result, a higher performance heat exchanger system was designed and built. At the same time, the thermode design was modified to make it easier to implant and protect.

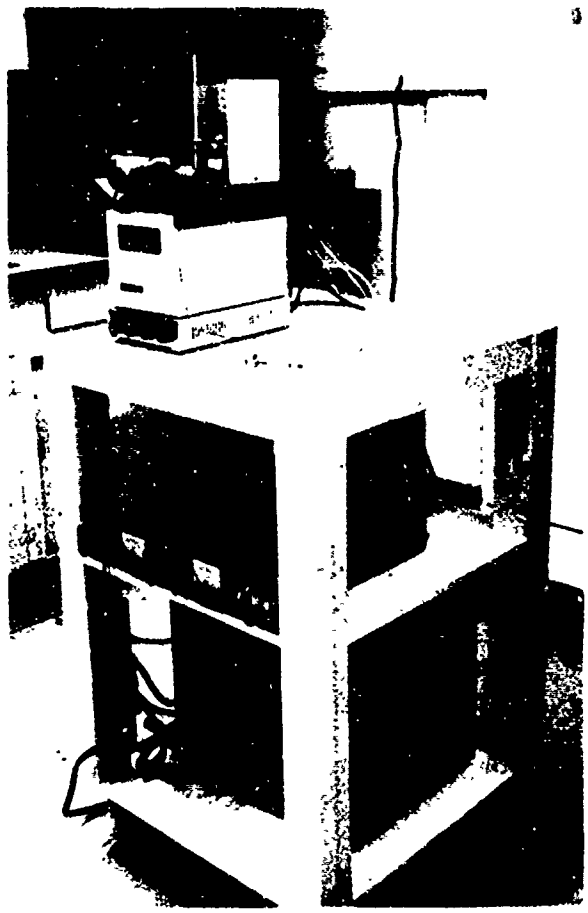

Fig. 1 Modified Thermode Design. (187515)

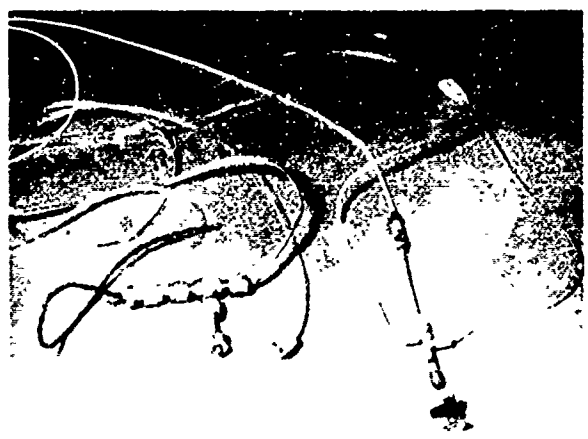

Fig. 2 Exploded View of Cortex-Implantable Cryoprobe Showing Thermode, Removable Heat Exchanger, and Thermistor Sensor. (187466)

The other design is shown in Figs. 1 and 2. A standard laboratory temperature-regulated cooling system is used to provide a reservoir

of temperature-controlled cooling fluid (methyl alcohol). Temperatures as slow as -40°C are possible under no-load conditions. The fluid is circulated through flexible lines to the heat exchanger using a small, variable-speed, high-pressure pump; flow rates through the heat exchanger can be as high as about 1.5 cm^3/s, giving a system time constant of about 2 s. An immersion heater placed in the fluid delivery line is used to modulate the heat exchanger temperature. The temperature is sensed with a thermistor probe located in the heat exchanger. The heat exchanger is built within a threaded copper slug that mates to the implanted thermode (see Fig. 2). Like the earlier design, the thermode is a composite copper/stainless-steel structure that is gold plated for chemical inertness. When implanted, the copper thermode will be in contact with the cortex, with the upper structure held rigidly to the skull with acrylic cement. The thermode is especially simple. Various shapes could be constructed to accommodate the topography of various areas to be cooled; all would use the common heat-exchanger assembly.

FUTURE PLANS

If evaluation shows this design to be successful, further funding will be sought in a separate grant proposal.

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING §52

Research by: F1C, FSE

Support: USPHS Grant NS-11710 (NINCDS), NIH Contract NS-5-2332-1, and APL Research Budget X85U

J. G. Chubbuck and L. J. Viernstein (APL), and A. E. Walker (JHMI)

October 1975-September 1976

INTRACRANIAL PRESSURE MONITOR

After neurological surgery, intracranial pressure is monitored so that emergency procedures can be instituted if it rises to dangerous levels. High intracranial pressures, if undetected and untreated, will seriously depress nervous system function and could lead to serious neurological deficits or possibly death. To provide reliable monitoring of intracranial pressure, APL is developing an instrument based on an implantable capsule containing a resonant tuned circuit. The principal parts of the instrument (Fig. 1) are the implantable pressure-sensitive capsule, an external detector, and a bedside monitor.

As part of the neurosurgical procedure, the capsule is placed in a burrhole, usually made for other reasons during the course of neurosurgery. The transensor is oriented so that the sensing diaphragm is resting on the dura mater. After the operation the resonant frequency of the sensor is measured by an external interrogator coil. The RF resonant frequency is compared with original calibration to give the magnitude of the pressure acting against the diaphragm. The bedside monitor will give a continuous recording and signal an alarm if abnormal intracranial pressures are detected.

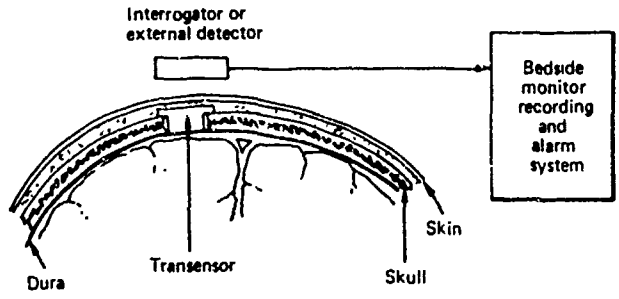

Fig. 1 Intracranial Pressure Monitoring System. (75-3/70)

SUMMARY AND CONCLUSIONS

Continued long-term drift problems have been encountered in the implants that have been used at several of the medical centers. While the APL implants are superior to other available implants, APL engineers believe greater stability can be achieved by changing the capsule design so that relative, rather than absolute, pressure is measured. Testing of a newly designed implant that measures relative intracranial pressure is now under way in animals. Meanwhile, patient monitoring with the capsules measuring absolute pressure is continuing at the University of New Mexico School of Medicine, Johns Hopkins Hospital, and the University of Southern California Medical Center.

DISCUSSION

The implant now used in patients is a passive device, consisting of electrical inductance and capacitance connected to form a resonant circuit in the VHF band. Pressure on the implant compresses an entrapped volume of gas, increases the capacitance, and thereby lowers the resonant frequency of the circuit. After implantation, the resonant frequency of the implant can be detected outside the body by placing in the vicinity of the transensor a coil radiating a swept-frequency VHF signal. The resonant frequency and hence the pressure on the implant are found by noting the frequency at which energy is absorbed from the externally located coil.

Like an aneroid barometer, the implant measures absolute pressure relative to the pressure of the gas within it. Hence the pressure readings made by the implant must be corrected for barometric pressure and temperature.

The principle of measuring intracranial pressure relative to absolute zero pressure assumes that the entrapped gas used as the pressure reference is very stable over a long period of time since changes in intracranial pressure are small compared to changes in atmospheric pressure. So far, the reference pressure has not been stable enough for long-term use of the implant. Another difficulty is that special implants must be constructed for high-altitude locations such as Albuquerque, NM. Such problems have led to the development of a new type of pressure-sensing implant that contains no entrapped gas and measures relative rather than absolute pressure.

Intracranial pressure is meaningful only relative to the surrounding atmospheric pressure. Therefore, an implant that directly compares intracranial pressure with atmospheric pressure not only eliminates the problem of a stable reference but also eliminates the effects of changes in atmospheric pressure and, to a large extent, in body temperature.

The differential-pressure-sensing implant that is presently being evaluated in dogs is shown in Fig. 2. A circularly cylindrical plastic (Lexan) enclosure, entirely filled with silicone oil, is separated into two chambers by a single nickel bellows and its ceramic support structure. The two ends of the plastic enclosure are very thin, forming two diaphragms able to transmit pressure from the exterior to the interior with very little attenuation. The lower diaphragm rests against the dura and transmits the pressure to the lower oil-filled chamber that includes the region surrounding the bellows. The upper diaphragm, lying outside the skull but below the scalp, transmits the subscalp pressure to the upper oil-filled chamber that includes the interior of the bellows. It is assumed that the subscalp pressure is an acceptable approximation of atmospheric pressure.

Deflection of the lower end of the bellows (closed end) will result from a pressure difference between the intracranial pressure and the

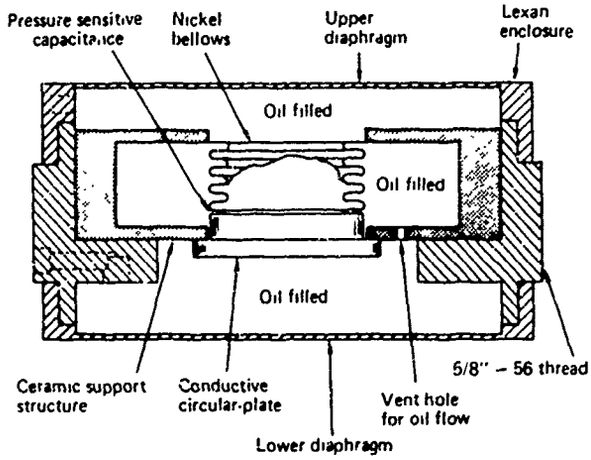

Fig. 2 Cross Section of the Differential Pressure Sensing Implant. (76-3/42)

subscalp pressure. The deflection will be proportional to the pressure difference multiplied by the area of the bellows and inversely proportional to the bellows spring constant.

The ceramic bellows-support structure also supports a rigid conductive circular plate close to the closed end of the bellows, forming a pressure-sensitive capacitance similar to that formed by the two bellows in the absolute-pressure sensing unit. One end of a coil wrapped around the outer surface of the ceramic support structure is connected to the bellows while the other end is connected to the conductive plate, forming a passive resonant circuit whose natural frequency is a function of differential pressure.

The lower chamber (Fig. 2) is the intracranial pressure side; hence the space between the bellows and the conductive plate will increase with increasing intracranial pressure. This choice places the most sensitive portion of the output range near normal intracranial pressure, and eliminates the upper bound on the pressure range that occurs in the absolute pressure unit when the two bellows touch at high pressure.

The differential sensor is easier to make than the absolute pressure sensor. For the absolute pressure sensor, the initial bellows spacing was controlled by mounting the two bellows in the two ceramic half-sections, which were then honed to a certain bellows spacing to set a particular resonant frequency. Cementing the two half-sections together increased the spacing by the thickness of the bond. In contrast, for the differential pressure sensor, spacing between the bellows and the conductive plate is adjusted by honing the conductive plate and then cementing it (with epoxy) to the ceramic support structure exterior to the mating surfaces of the plate and the support structure. This technique makes the initial frequency setting more predictable.

A vacuum technique is used to fill the differential sensor with oil after the plastic enclosure (except for closing the vent holes shown in the figure) is assembled. The sensor is placed in a high-vacuum chamber where it is submerged in silicone oil. When the vacuum is released, both chambers are completely filled with oil. The gas entrapment feature of the absolute pressure sensor did not permit this technique to be used.

FUTURE PLANS

Six of the pressure-sensitive implants that respond to relative pressure are now being tested in four dogs. If reliability requirements are met and the implants for relative pressure prove more stable than the implants for absolute pressure, the fabrication process at APL will be converted to make the new implant in quantity. All the medical centers will then be supplied with the new implant. Work will also continue on developing an improved bedside monitoring instrument that will provide continuous recording of intracranial pressure and signal alarms if abnormal intracranial pressures are detected.

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING §51

Research by: RTP and JHMI
Support: USPHS Grant HL-19095

R. L. McCally and E. A. Michelson (APL), and S. Margolis (JHMI)

June-September 1976

LASER SPECTROSCOPY OF LIPOPROTEINS

This research is directed toward determining in what ways the size distributions of low- and very-low-density lipoproteins (LDL and VLDL) differ among carefully chosen members of families having classical Type II and Type IV hyperlipoproteinemia, among normals in the general population, and among normals in the above families. We also intend to investigate the structural importance of certain components of LDL by chemically modifying them and observing the effect of these modifications on the diffusion coefficient and its variance.

The principal technique used in the research is intensity correlation spectroscopy (ICS). ICS is now the most rapid and accurate method of obtaining macromolecular diffusion coefficients. In addition, it can provide valuable measures of polydispersity.

SUMMARY

The Public Health Service initiated funding to support this research for three years on 1 June 1976. Since that time we have been performing experiments to discover ways to reduce the number of small aggregates in normal LDL solutions (which we had noted earlier) or to understand their cause (Refs. 1 and 2). In this regard, we have begun experiments to determine (a) the effect of sample aging on the diffusion coefficient and its variance; (b) the effect of multiple centrifuge spins during LDL isolation on its final purity (particularly contamination by VLDL); and (c) the effect of 8 M urea on LDL size and polydispersity. Also the auxiliary electronics and new photomultiplier for the photon-counting correlator have been purchased. The construction of the new correlator is expected to begin in October.

FUTURE PLANS

The experiments to discover the source of aggregates in normal LDL solutions will be completed. The new correlator will be constructed and incorporated into the apparatus. Size distribution studies on fresh, normal VLDL will be initiated, and the light-scattering results will be compared to electron microscopy.

BACKGROUND

Lipoproteins are of considerable biological interest since they play a major role in the transport of lipids throughout the body. LDL are the most abundant lipoproteins in human plasma and are the major carriers of cholesterol. VLDL are the main carriers of triglycerides. Operationally, lipoproteins are defined by their flotation properties in the ultracentrifuge. LDL comprise those lipoproteins in the density range 1.019 to 1.063 gm/cm^3, whereas VLDL have densities below 1.006 gm/cm^3 (excluding the transient species called chylomicrons that appear shortly after eating and disappear within a few hours). Chemically, LDL contain 75 to 78% lipid, 20 to 22% protein, and 3 to 5% carbohydrate, and VLDL contain approximately 89% lipid, 10% protein, and 1% carbohydrate. Both LDL and VLDL are polydisperse. Electron micrographs show that both are spherical, that LDL diameters range between 19 and 25 nm, and that VLDL diameters range from 30 to greater than 90 nm (Refs. 3 and 4).

In our research we are examining the species using the ICS technique which is presently the most rapid and accurate method of obtaining macromolecular diffusion coefficients and their variances (Refs. 5 and 6). Diffusion coefficients are important in the study of macromolecules because they are related to the size of the molecule in solution by the Stokes-Einstein relation (Refs. 5 and 6). The diffusion coefficient is determined from the autocorrelation function of the time-dependent intensity fluctuations in the light scattered from the particles as they diffuse in solution. The general form of the correlation function is $C(\tau) = A|g^{(1)}(\tau)|^2 + B$, where A, B, and τ are the amplitude, background, and delay time, respectively. For a single-sized molecular species, $g^{(1)}(\tau) = \exp(-Dq^2\tau)$, where D is the translational diffusion coefficient. The factor q^2 is an instrumental parameter equal to $(16\pi^2n^2/\lambda_0^2)\sin^2(\theta/2)$, in which n is the refractive index of the solvent, λ_0 is the vacuum wavelength of the incident laser radiation, and θ is the scattering angle.

For polydisperse samples, the interpretation of data is more difficult. Two different approaches can be taken. The cumulant method (Ref. 5 and 6) provides a completely general description of polydisperse systems. In this method, $\ln g^{(1)}(\tau)$ is expanded as a truncated Maclaurin series in τ:

$$\ln g^{(1)}(\tau) = \tfrac{1}{2}\ln\left[\frac{C(\tau) - B}{A}\right] = -\tfrac{1}{2}\ln A + \sum_{m=1}^{M} K_m^M \frac{(-\tau)^m}{m!} . \quad (1)$$

The K_m^M are the cumulants and, in most experiments, M is limited to 2 or 3 by noise on $C(\tau)$. The first cumulant, K_1, is related to

the z-average diffusion coefficient of the scatterers by $K_1 = \bar{D}_z q^2$; K_2 is related to the variance of the distribution of diffusion coefficients; and K_3 is related to the skewness (Refs. 5 and 6). The second approach, which is less general than the method of cumulants, is to specify a form for the distribution of molecular sizes (or, equivalently, diffusion coefficients) and to obtain the values of the parameters of this specified distribution from the correlation function or from the cumulants (Refs. 2 and 7). Both approaches are proving useful in studying LDL and VLDL (Refs. 1 and 2).

DISCUSSION

Our preliminary experiments on LDL, prior to NIH funding, suggested that small aggregates were present in the solutions even after the extensive filtration and centrifugation required to prepare the samples for light-scattering measurements (Refs. 1 and 2). We based this conclusion on the fact that our measured diffusion coefficients (extrapolated to zero concentration) were about 6 to 10% lower than previously reported values and that the value of the reduced second cumulant, K_2/K_1^2 (which is also the variance of the distribution of diffusion coefficients), indicated a greater degree of polydispersity than was shown in electron micrographs (Refs. 1 and 2). The early results were consistent with a monomer-oligmer equilibrium; however, they were generally obtained on samples over a period of several weeks after preparation. Also, the early samples were centrifuged only once at each salt density during their preparation. Therefore we set out to determine the effect of sample aging on the diffusion coefficient and to see if sample purity is improved by spinning the sample twice at $\rho = 1.019$ gm/cm^3 (this step would reduce the possibility of contamination by larger VLDL particles). We also wanted to see if placing the LDL in 8 M urea would have an effect on the aggregation (8 M urea is a commonly used "disaggregating agent" in protein research, but its effect on LDL has not been reported).

The effect of aging on the LDL samples that were spun twice at 1.019 gm/cm^3 is shown in Fig. 1. The 5 mg/ml sample changes much less rapidly with time than the 1 mg/ml sample. The value of $D_z^{20°}$ obtained for the 1 mg/ml sample is within about ±1% of previously reported values (Ref. 8) and corresponds to an average diameter of 22.5 nm. Apparently, less concentrated LDL samples can be safely used only up to about one week after isolation, and more concentrated samples appear to have degraded only slightly after two weeks. The experiment to determine the effect of multiple spins during sample preparation on sample purity was less conclusive; however, $D_z^{20°}$ of a single-spin 5 mg/ml sample was 3% lower than that of the two-spin sample when measured on the same day. Urea has a profound effect on the size of LDL. After correcting the diffusion coefficient for the viscosity of the 8 M urea solution, we find that, one week after isolation, the apparent diameter of LDL in a 1 mg/ml solution is approximately 38 nm. Qualitatively, the effect of concentration appears similar to that in standard solutions, but the effect of LDL aging appears greater in urea.

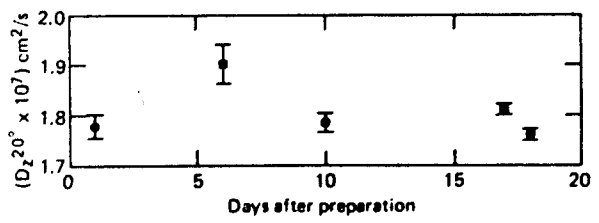

Fig. 1 Effect of Age on LDL Diffusion Coefficients.
▪ 1 mg/ml sample and • 5 mg/ml sample (76-3/46)

The values of the variance of the distribution of diffusion coefficients appear to be substantially less than obtained previously (approximate values ranging from 0.07 to 0.09 were found versus the 0.11 to 0.16 obtained for earlier samples). This suggests that our present samples were much less polydisperse than the older samples used in our preliminary studies. However, analysis of the data for this quantity has been difficult because the Saicor correlator seems to be intermittently adding nonrandom noise to the data (this is a small effect and does not influence the values of K_1 and hence D_z). The photon-counting correlator, which will be completed in the coming year, is expected to eliminate such difficulties and to improve markedly our ability to measure this important parameter.

REFERENCES

1. C. B. Bargeron, R. L. McCally, and M. H. Friedman (APL) and S. Margolis (JHMI), "Particle Size Distributions of Human Plasma Lipoproteins by Intensity Correlation Spectroscopy," Biophys. J., Vol. 15, Part 2, 1975, p. 251a.
2. Section 54 (Unclassified), Quarterly Report, January-March 1975, APL/JHU C-RQR/75-1 (Confidential).
3. D. S. Fredrickson, "Plasma Lipoproteins and Apolipoproteins," Harvey Lectures, Academic Press, New York and London, 1974.
4. V. T. Schumaker, "Hydrodynamic Analysis of Human Low Density Lipoproteins," Accts. of Chem. Res., Vol. 6, 1973, p. 398.
5. P. N. Pusey, "Macromolecular Diffusion," Photon Correlation and Light Beating Spectroscopy (H. Z. Cummins and F. R. Pike, eds.), Plenum Press, New York, 1974.
6. D. E. Koppel, "Analysis of Macromolecular Polydispersity in Intensity Correlation Spectroscopy: The Method of Cumulants," J. Chem. Phys., Vol. 57, 1972, p. 4814.
7. C. B. Bargeron, "Measurement of a Continuous Distribution of Spherical Particles by Intensity Correlation Spectroscopy: Analysis by Cumulants," J. Chem. Phys., Vol. 61, 1974, p. 2134.
8. R. W. DeBlois, E. E. Uzgiris, S. K. Devi, and A. M. Gotto, Jr., "Application of Laser Self-Beat Spectroscopic Technique to the Study of Solutions of Human Plasma Low-Density Lipoproteins," Biochem., Vol. 12, 1973, p. 2645.

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING § 52
Research by: BTT, RAM, REP, and RTP
Support: USPHS Grant HL-14207 (NHLI)
C. B. Bargeron, O. J. Deters, L. W. Ehrlich, F. F. Mark, V. O'Brien, and M. H. Friedman
October 1975-September 1976

PULSATILE FLOW STUDIES

The broad objective of this program is to evaluate several hypotheses implicating fluid mechanical effects in the etiology of atherosclerotic disease. This requires knowledge of how the hypothesized hemodynamic determinants of atherosclerosis vary through the arterial tree. Because in vivo physiological experiments are very difficult, computational and experimental simulation models are used.

SUMMARY

Included in this report are computations of particle paths and stasis in a Y-shaped two-dimensional symmetric branch, experimental measurements of velocities near and parallel to the outer wall of a branch with unequal flow partition, and computed values of the velocity in a branch with a slightly rounded corner. The computations of particle paths are based on previous fluid mechanical simulations of pulsatile blood flow that provide the time-dependent velocity field (Refs. 1, 2, and 3). In regions where separation occurs, the particles trace paths that cannot be predicted by a steady-flow calculation. No particle returns to its starting point in one pulsatile cycle. An occlusion in a branch produces significant changes in the velocities near the walls and corresponding variations in wall shearing stresses. The effect of slightly rounding the corner is to increase the value of the minimum velocity that occurs immediately downstream of the corner, in better agreement with experimental results.

DISCUSSION

Among the sites in the vascular tree at which atherosclerotic lesions seem predisposed to form, a substantial number might be expected from fluid mechanical considerations to be sites of low shear, separation, or stasis. Indeed, all three phenomena have been proposed as being responsible, at least in part, for the distribution of early vascular lesions. However it should be noted that stasis does not have a precise definition, although shear and separation can be defined. Stasis is a Lagrangian property, associated with particle trajectories, and cannot be measured by many techniques conventionally used to probe the Eulerian flowfield. This distinction is particularly important for the unsteady pulsatile flows of principal interest here. In steady flow, fluid particles remain on streamlines; if the streamlines are closed, as in a separation bubble, the particles in principle never leave the region. In contrast, when the flow is unsteady, the velocity field changes with time and the particle does not follow any single streamline.

In the studies reported here, the flowing blood is regarded as homogeneous, and the calculated particle trajectories are those of "fluid particles" since our understanding of the interaction of hydrodynamic and inertial forces in fluids of high particle density (hematocrit) is simply insufficient to calculate the trajectories of formed elements. Emphasis is placed on fluid particle dynamics in unsteady flow through a two-dimensional Y-branch (Fig. 1), where the nonlinear terms in the Navier-Stokes equation must be retained and the streamline representation appropriate to the description of particle trajectories in steady flow cannot be used.

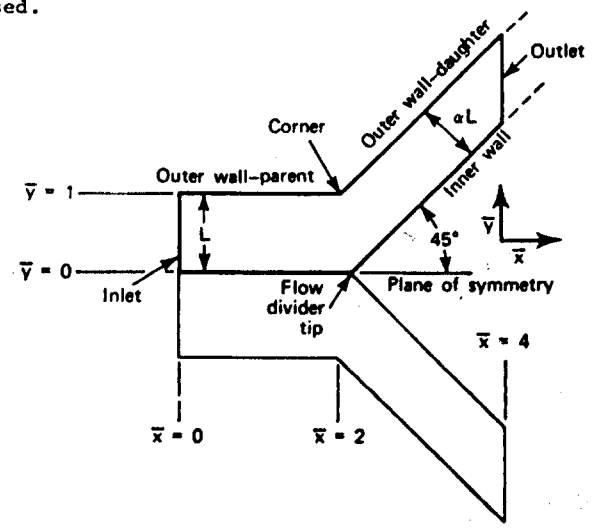

Fig. 1 Y-Branch Geometry. (189829)

To determine particle paths, it is necessary to have a complete velocity vector field. The computational scheme described in Ref. 4 yielded the stream function only at grid points. Thus, these data were splined using two-dimensional cubic splines that were then differentiated to obtain velocity vectors. When particles crossed the outlet of the branch, their trajectories were continued using the analytic solution for fully developed flow in a plane-parallel channel. The flowfield used was for a simple pulsatile flow with a mean Reynolds number based on parent channel half-width of 100 and an unsteadiness number $\eta = 10$ (Ref. 5).

When the pulsatile flow is passed through the branch, a region of transient separation develops distal to the corner, along the outer wall of the daughter, that lasts a little over 10% of the cycle. The maximum extent of the region will be termed the "separation area." Outside the separation area, the flow is such that most fluid particles in the daughter either are swept out of the bifurcation in much less than one cycle or, if they start close to the wall, travel nearly parallel to the wall for a distance similar to that which they would travel in a straight channel. The particles that are of the greatest interest are those that start near or in the separation area. Our attention was thus directed to this part of the branch.

The distance a particle travels during a pulsatile cycle is a function of both the starting position and the time during the cycle at which the position is occupied, i.e., the starting time. If a particle originates distal to the separation area, it travels more or less parallel to the wall. On the other hand, if the particle starts in the separation area, its final position depends upon whether or not separation occurs while the particle is there. Particles that start in the separation area nearer zero flow parallel the wall and do not travel far. However, near maximum flow, the particle travels in a swirling path and can be swept out

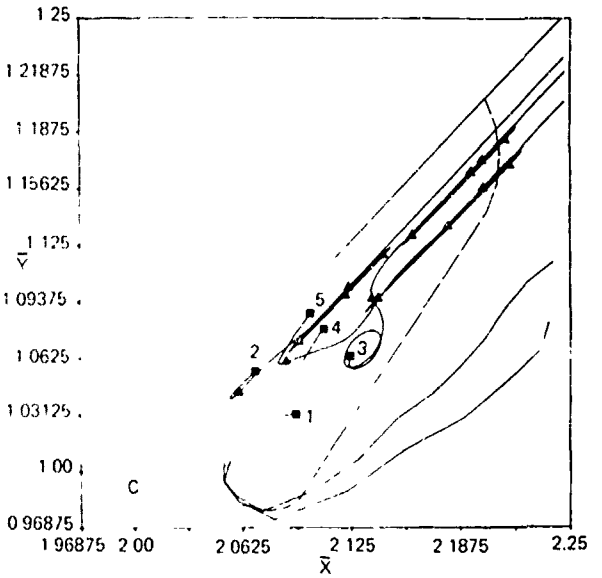

Fig 2 Trajectories of a Set of Particles Starting Inside the Separation Area — —When the Flow through the Branch Is at a Maximum. At maximum flow, the separation area is fully occupied by a separated flow. ■ . starting point; ▲ : position of particle at time intervals of 1/8 of a pulsatile cycle. (189828)

of the area into the rapid mainstream flow and carried a considerable distance.

The trajectories of particles starting within the separation area depend on where in the area they start. Figure 2 shows the paths of several particles originating in the separation bubble at maximum flow. The paths vary considerably and bear no resemblance to the closed streamlines followed by a particle in a steady separation bubble. Paths 1 and 4 are illustrative of the trajectories of a large fraction of the particles originating in the bubble; the circulation in this region causes the particle to move out towards the mainstream which sweeps it away. Particles starting close to the wall (paths 2 and 5) move more slowly and do not reach the mainstream before the separation bubble dissipates. The particle that follows path 3 appears to have originated near the vortex center. As with particles originating outside the separation area, no particle examined here returns to its starting position; all proceed downstream. This observation implies the absence of "perpetual" stasis in the pulsatile flow case.

Further experimental studies have been made to supplement the previous studies reported in Ref. 3. Measurements were initiated to define the effects of unequal flows through the daughters of the Y-branch. The calculation of the flowfield for this situation is extremely costly and hence represents one of those aspects of hemodynamics better suited to experimental study. Initial measurements of the steady flow velocity parallel to the left wall of the channel and 0.038 cm from the wall were made first with the right branch partly occluded and then with the left partly occluded. A comparison of the flow velocities when each branch is occluded with the flow velocities for equal flow in the branches is given in Fig. 3. Significant increases and decreases in the velocities at the corner are evident with corresponding variations in the shearing stresses at the walls; these changes may be of importance in the hemodynamics of arteriosclerosis. Further experimental velocity measurements in pulsatile flow with the branches occluded are expected to show similar characteristics.

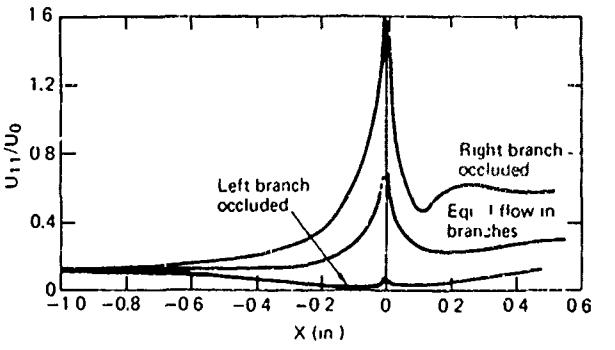

Fig 3 Measured Velocity Component Parallel to and 0.015 in. from the Left Wall for Steady Flow at Reynolds Number of 230. With the right branch partly occluded, 98% of the flow passes through the left branch; with the left branch partly occluded, 27% of the flow passes through the left branch. (76 3/37)

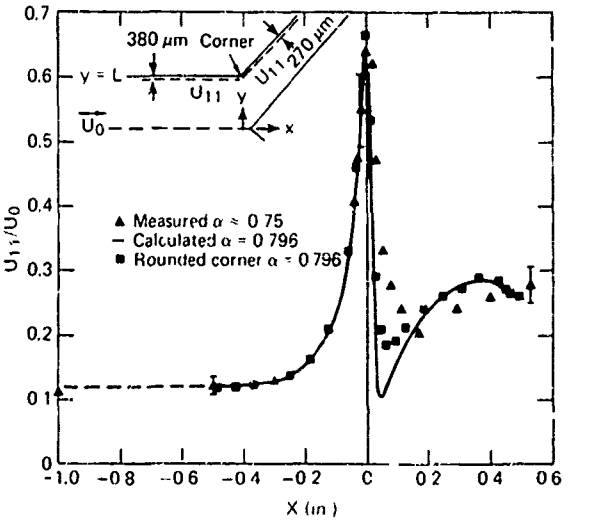

Fig. 4 Comparison of the Measured Component Parallel to and 0.015 in. from the Wall with the Corresponding Numerical Calculations in Steady Flow with and without the Rounded Corner (76-3/38)

A feature of the steady flow computations is a sharp minimum in the velocity near the wall immediately downstream of the corner. The usual close agreement between experimental measurements and the computed velocity field was not evident with regard to either the position of, or the value of the velocity at, this minimum. A possible cause of the disagreement was believed to be a slight rounding of the corner in the experimental model. When the corner in the computational model is similarly rounded, the calculated minimum is shifted upward and slightly downstream in closer agreement with the experimental results (Fig. 4).

REFERENCES

1. Section 143, Annual Report, October 1972-September 1973, APL/JHU MQR/73-3.
2. Section 98, Annual Report, October 1973-September 1974, APL/JHU MQR/74-3.
3. Section 75a, Annual Report, October 1974-September 1975, APL/JHU MQR/75-3.
4. Section 129, Annual Report, October 1971-September 1972, APL/JHU MQR/72-3.
5. M. H. Friedman, V. O'Brien, and L. W. Ehrlich, "Calculations of Pulsatile Flow through a Branch: Implications for the Hemodynamics of Atherogenesis," Circ. Res., Vol. 36, 1975, pp. 277-285.

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING §53
Research by: REM, RCO
Support: Richard King Mellon Foundation
A. B. Fraser and C. D. Mitchell
December 1975-September 1976

TOMOSYNTHESIZER

The tomosynthesizer provides a way to reconstruct any tomographic plane from a single set of 20 X-ray projections (Refs. 1 and 2). Thus, the tomosynthesizer is a "3D X-ray" device. In the tomosynthesizer, 20 radiographic projections made with a stationary polytome are illuminated simultaneously. A lens and mirror arrangement directs the light attenuated by the radiographs along paths that are exact replicas of those originally traversed by the X-radiation. The intensity of light reaching any point in the volume of overlapping rays from the projections is an estimate of the X-ray absorption at that point. The user of the tomosynthesizer produces visible output images of the X-ray attenuation on planes in the X-rayed volume by manipulating a light-scattering screen to the planes that are of interest.

The tomosynthesizer was built a few years ago, and reconstruction of tomographic planes was demonstrated by partly manual means. Automatic equipment to facilitate production of the radiographs and to "inject" them into the tomosynthesizer was nearly completed at that time. With the present Mellon Foundation grant, we are completing the automation of the device, are testing it, and will soon be clinically evaluating it in collaboration with Dr. G. Saba of the Radiology Department at JHMI.

SUMMARY

A camera for recording the 20 original X-ray projections on 70-mm film was modified optically and mechanically and tested. The output of the X-ray camera passes through two other automatic devices, the injector and the disk cassette, to produce a circular disk of 20 projections on photographic film. The projections are later reprojected to bring about the tomosynthesis. The optics of the injector and the mechanism of the disk cassette were reworked and tested. The three devices have been used successfully with the polytome and the tomosynthesizer to reconstruct planes of X-ray absorption in test objects and in phantoms.

Convenient use of the tomosynthesizer requires accurate and easy positioning of the bar mechanism of the polytome. This mechanism (weighing a few hundred pounds) must be positioned with high precision at 20 equally spaced positions for accurate tomosynthesis. To allow such positioning, a 20-stop, 14-in.-diameter geneva mechanism was built (Fig. 1). It positions the polytome yoke within 15 arc-seconds of the 20 stops ideally required. The mechanism gradually accelerates and decelerates the polytome's bar mechanism, resulting in smooth, silent operation. Circuits connected to the mechanism automatically advance the film in the X-ray camera. They also indicate locked stops for exposures.

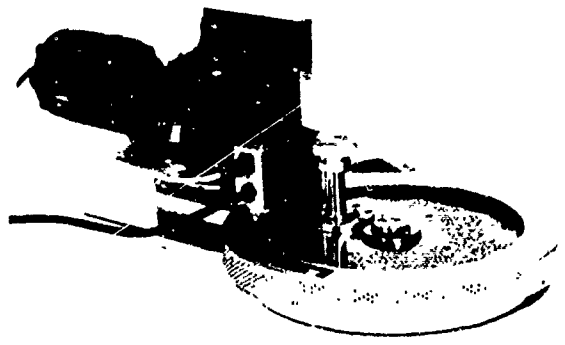

Fig. 1 The Geneva Mechanism. The black motor at the left turns continuously, and the geneva mechanism generates an intermittent motion at the vertical drive pin. The drive pin, with the knurled retainer above it, smoothly moves between 20 predetermined positions and thereby indexes the polytome. (191750)

To reduce X-ray exposure, always an important parameter in radiological examinations, more sensitive photographic procedures have been developed. Presently, adequate resolution and contrast are being attained at an ASA speed of 8000 and a film exposure of 10^{-9} W-s/cm^2.

A better light scattering screen for the tomosynthesizer was installed. Light strikes the reconstruction screen at various angles and proceeds to the viewer at a wide range of scattering angles from the incident light paths. Optimum reconstruction of tomographic planes requires that equal weight be given to the light from each of the projections. The closest one can come to this ideal is with a Lambertian scattering surface, which produces a scattered intensity proportional to the cosine of the scattering angle. A special-order commercial screen called "Lenscreen TR 50," which produces a nearly Lambertian scattering function, was installed in the tomosynthesizer and the injector. Reconstructions with the "TR 50" screen showed a much more even summation of the projections, a high resolution, and even greater brightness than previous screens provided.

A device we call the "flicker" was built and installed in the tomosynthesizer. The flicker sequentially covers each of the projections at a rate of about 0.1 s per projection. A person using the tomosynthesizer with the flicker operating sees that some of the detail on the reconstructed plane "flickers" as the flicker covers and uncovers the projections singly and serially. Any detail that flickers is not from X-ray attenuation in the reconstructed plane; X-ray attenuation in the plane of reconstruction exists on all contributing projections equally and does not flicker. Thus, the flicker provides a convenient way to allow the user's eye and brain to discriminate the origin of X-ray density appearing on tomographic planes. Figure 2 shows photographs of the reconstruction screen made with the flicker in three sequential positions of its traverse. Figures 2a through 2c show a sequential disappearance of X-ray information arising from a dense sphere that was out of plane.

FUTURE PLANS

Clinical investigation of the tomosynthesizer will begin soon. We foresee possible tech-

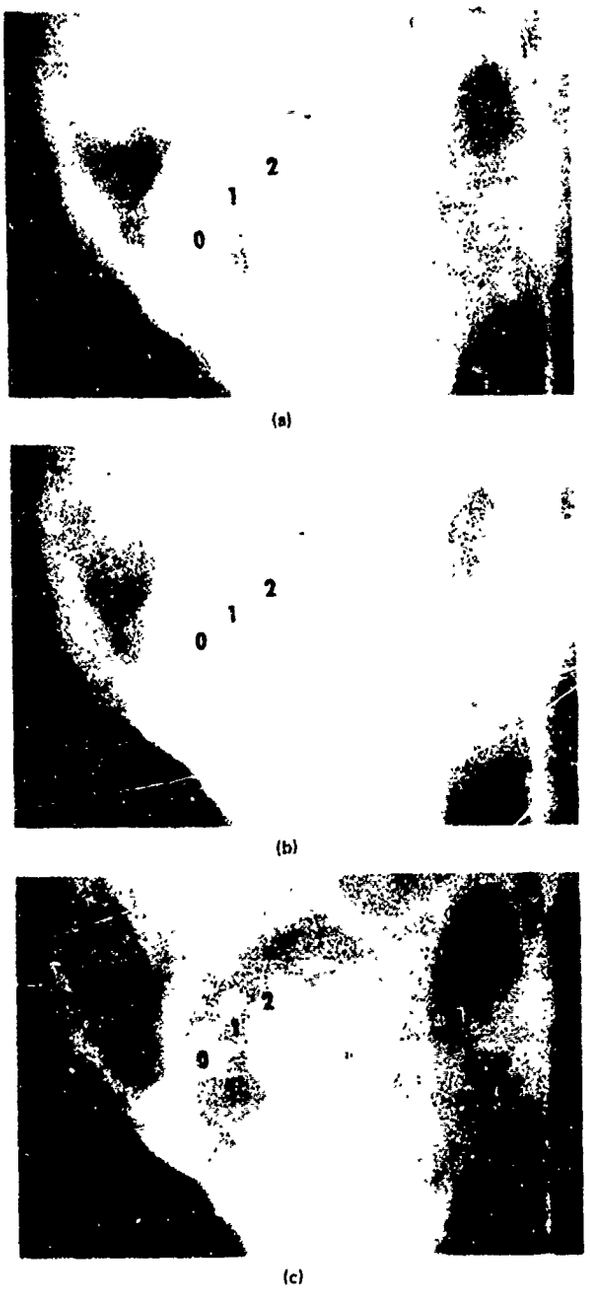

Fig 2 The Use of the Flicker. Still pictures were made with the flicker at three sequential positions. The numbers 0, 1, 2 are immediately below three positions where information from an out-of plane dense sphere appears The three shadows disappear with the sequential flicker positions. (191749)

nical improvements. Reduced X-ray exposure may be possible through additional refinements in photographic technique and through the introduction of a multichannel plate image intensifier in the X-ray camera. More effective processing of tomographic projections in the user's eye and brain may be possible if a color filter analog of the flicker is used. We intend to proceed toward the goal of an X-ray tomographic device that will reconstruct all tomographic planes with roughly the X-ray exposure required to resolve one plane. Each reconstructed plane should provide better rejection of out-of-plane information than is available by classical tomography.

REFERENCES

1. J. B. Garrison, D. G. Grant, W. H. Guier, and R. J. Johns, "Three Dimensional Roentgenography," Am. J. Roentgenology, Radium Therapy and Nuclear Medicine, Vol. CV, No. 4, April 1969, pp. 903-908.
2. D. G. Grant, "Tomosynthesis: A Three Dimentional Radiographic Imaging Technique," IEEE Trans. Biomedical Engineering, Vol. BME-19, No. 1, January 1972, pp. 20-28.

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING §54
Research by: FSO and FIC
Support: Veterans Administration Research Contract W. Seamone (APL) and G. Schmeisser, Jr. (JHMI)
October 1975-September 1976

DEVELOPMENT AND EVALUATION OF A POWERED MEDICAL MANIPULATOR

A major portion of the APL effort on the powered prosthesis and orthosis research program during the past year was concentrated on developing and evaluating the powered medical manipulator. This device will provide some assistance to the highly disabled person, such as a quadriplegic, who is totally reliant on others for all his needs.

Two experimental manipulator systems were designed and fabricated for clinical trials to obtain basic data on the value of such a system in a patient environment. This progress report summarizes the results of evaluation of the first system by two patients.

SUMMARY

This research continues a multiyear effort to develop powered prosthetic and orthotic systems for the Veterans Administration. A wide range of patient requirements is included in the study. The program is a collaborative APL/JHMI effort of engineering and clinical evaluation to develop hardware designs of practical value to the handicapped individual. A powered medical manipulator based on concepts and component designs from powered prosthetic systems that were previously developed is currently undergoing clinical evaluation. Two patients have been included in the clinical program to date. The first patient interfaced the system by means of a headstick control. Results of his evaluation were reasonably satisfactory; however, when the patient was transferred out of the Washington, DC, area, tests were discontinued and additional modifications were made to the system. After suitable design modifications, a second patient was selected who has been operating with the system for over seven months. He has demonstrated that the powered medical manipulator can significantly assist him in doing certain tasks. These tests are being continued. As a result of the tests, two more systems were requested by the Veterans Administration for clinical evaluation in VA hospitals. One unit has been delivered to the VA Hospital, Castle Point, NY, and a second is planned to be completed in October 1976 for delivery to a VA Hospital in Boston, MA.

DISCUSSION

After about 150 h of manipulator use at the Maryland Rehabilitation Center by a middle-aged, male quadriplegic with a total neurological deficit below C_4, it was recognized that the manipulator reached its maximum ease of operation, efficiency, and usefulness when integrated into a worktable arrangement with appropriate vocational tools. The patient's performance with the system was optimal when the equipment was arranged to exploit a skill that the operator already possessed rather than to attempt to substitute for this skill with the manipulator. The patient worked primarily with the typewriter and made some unsatisfactory attempts at self-feeding. He was then transferred out of the Washington area and his clinical evaluation terminated. Much information was gained from the initial tests, and the equipment was then modified to increase its flexibility. Some of the new features shown in Fig. 1 include:

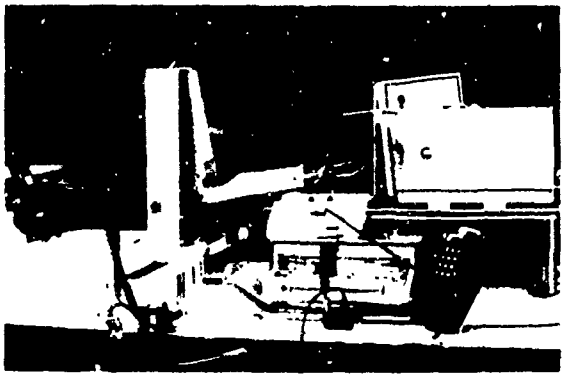

Fig. 1 Mod 1A Manipulator/Worktable for VAPC, Castle Pt., NY. (190708)

1. New Control Mode Options. A choice is available between direct keyboard operation for mode select with the mouth stick, or automatic sequence, stopping on pulse command.
2. Chin Nudge Control. A chin nudge transducer was added to effect proportional control of the desired motion of the manipulator. The transducer is attached to the worktable and may be swiveled in or out of contact with the chin by the patient's head motion. Good proportional control is achieved by the transducer.
3. Telephone Option. For those patients who desire or need to use the telephone, this option is provided. All telephone components are unmodified, and the system employs a standard hand-held receiver. A Touch-Tone module is suitably located on the worktable to allow the patient to dial with his mouth stick. The handset is swung to the patient's head with the manipulator when making or receiving a call.
4. Two-Length Mouth Stick. A suitable holder was provided on the worktable for the mouth stick. The patient may retrieve or store the mouth stick in this holder as desired. The mouth stick is made in two pieces with a magnetic coupling inside an aluminum sleeve. The longer length, with the rubber tip, is generally used for reading. The shorter length is used to Touch-Tone dial, to type, and for direct keyboard control of the manipulator.

The manipulator and integrated worktable with these advanced features were fitted to a high-level quadriplegic in a nearby nursing home on 22 January 1976.

The individual has used the Mod I unit 2 to 4 h each day for the past seven months. He

Fig 2 Patient Uses Manipulator to Bring Food to His Mouth. (190706)

has evolved from originally using it primarily to evaluate its various features for research purposes to presently depending on it to support himself financially in reviewing and preparing technical reports in his profession as a consulting physicist for a Government agency. The system permits him to select items of literature of diverse sizes and physical characteristics from a file, to position them for examination, and to turn the pages forward and backward. Using a standard electric typewriter, he can type out appropriate letters and technical reports on the literature. By using the manipulator to position the standard telephone handset, he receives and places telephone calls directly and without assistance. He has become an enthusiastic advocate of the equipment, performing demonstrations and answering numerous inquiries from interested persons.

To facilitate self-feeding with the manipulator, a unique eating utensil has been designed, fabricated, and subjected to clinical trial. This accessory consists of a "spork" (combination spoon/fork) gimballed to swing (roll) sideways. It is attached by the attendant to the terminal device when food is brought to the patient. He has performed some trial attempts at eating a meal with the spork.

Although the patient can eat an ordinary meal with the manipulator/utensil combination, as shown in Fig. 2, the requirements of consciously commanding and controlling each motion of each joint of the manipulator during each plate-to-mouth (and return) portion of the feeding cycle are too tedious to be practical. To overcome this difficulty, an automatic sequence program whereby the appropriate sequence of joint motions is preprogrammed (but the velocity and range of motion are still controlled by the chin nudge transducer) has been developed, and some clinical testing has been performed. Experience to date indicates that additional work in preprogramming may be required to make self-feeding practical. Other common operations, such as fetching the typewriter from its stored position and loading it with paper, or fetching reading materials from the file to the reading stand, could also be expedited by appropriate preprogramming.

During FY 76, one powered manipulator/worktable was designed and fabricated similar to the JHU experimental model for delivery to the VA Hospital in Castle Point, NY. This unit (Mod IA) will undergo limited clinical evaluation to get basic data on its value to the patient with a cervical injury.

The layout of components and the basic design are similar to the model undergoing evaluation (Mod I) described herein. The electronics for this model are on a plug-in card located inside the vertical post. If an electronic malfunction occurs, the plug-in board can easily be replaced. Experience to date on the Mod I system indicates that the electronic controls for the manipulator have been highly reliable and very few failures have been experienced. The Mod IA was delivered on 2 June 1976, and evaluation tests will be conducted by the Veterans Administration Prosthetics Center after the protocol is developed.

FUTURE PLANS

Additional clinical testing is planned for the powered medical manipulator. System design improvements will be made as clinical testing progresses. Tests to date show promise for this device. Additional tests with several patients in different clinical environments should help validate the need for such units for more general clinical use.

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING §55
Research by: F3C
Support: The Johns Hopkins University
B. I. Blum
October 1975-September 1976

SUPPORT TO OFFICE OF HEALTH CARE PROGRAMS

As part of the ongoing APL responsibility in Clinical Information Systems, support is provided to the Johns Hopkins Hospital Office of Health Care Programs (OHCP). Activities include general participation in the analysis of information and information requirements plus specific responsibilities in selected programs. During the reporting period, APL was tasked to support the implementation of a stand-alone, professional fee financial system and to modify and improve the operation of the Minirecord System (see the previous Annual Report).

DISCUSSION

Support to the OHCP falls into three categories. The first, general support, involves APL participation in OHCP activities. A major activity during the past year has been to establish an Outpatient Department (OPD) System committee that is responsible for developing system and data requirements for an OPD System over the next few years. Features of the system may include on-line OPD registration, an automated appointment and scheduling system, expanded use of the Minirecord System, an on-line patient identification system, etc. APL activities have involved working with the committee to develop the specifications and working with physicians and major OPD clinics to define user requirements. The development of formal specifications and an implementation plan is scheduled for the next reporting period.

A second category of support involved the implementation of a professional fee financial system. This function was being performed by a service bureau located in the Midwest. After working with the system for some time, it was decided that the same function could be performed more efficiently if there were a local system using on-line terminals with an interface to the central JHH financial system. As a result, a turnkey system was procured and installed.

The APL involvement in the transition from the old to the new professional fee system included consulting plus conversion of machine-sensible records from the service bureau files to the stand-alone system. Included in the conversion process were preparation of reports, identification and flagging of errors, and merging and reorganization of data.

The third category of support centered around the enhancement of the Minirecord System (see the previous Annual Report). The system was developed by APL as an IR&D project and accepted by the Hospital as an operational system. After one year of operational use, the system was evaluated and the following facts were noted:

1. The availability of a minirecord has a significant effect upon an encounter,
2. The use of minirecords was growing, and
3. The updating of minirecords was difficult and thus led to out-of-date information.

As a result of the study, a committee was formed to redefine the system flow to facilitate the update process. The committee was charged with developing a preprinted encounter form that would contain the minirecord problem lists and medications, and identifying system changes required to implement the form. Because of the OPD System definition activity, it was agreed that modifications to computer programs would be held to a minimum.

APL provided the Project Engineer for the minirecord upgrade. Responsibilities included correction of patient identification inconsistencies among files, coordination of the definition of a new encounter form among the major users, specification of computer program changes, assistance in the documentation of operating procedures and training materials, and preparation of a formal proposal to OHCP. Completion of the proposed modifications is scheduled for the next reporting period.

FUTURE PLANS

During the next year, general support will continue in each of the above categories. The Minirecord System changes will be completed, and attention will be directed to the expansion of the minirecords to other clinics. The major follow-on activity will be to define OPD System requirements.

REFERENCE

1. C. J. Johns, B. I. Blum, and D. W. Simburg, "The Minirecord Approach to Continuity of Care for a Large Population of Ambulatory Patients," Proceedings, Third Illinois Conference on Medical Information System, 1976.

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING §56

Research by: CBP, F3C, and S1A

Support: Johns Hopkins Cancer Center, The Johns Hopkins University, and APL

W. H. Guier, B. I. Blum, H. D. Black, and J. B. Oakes

October 1975-September 1976

CANCER CENTER COMPUTING SYSTEM

APL has provided help to the Johns Hopkins Cancer Center (JHCC) in identifying and specifying requirements for a comprehensive data processing system to support the Center. The system will consist of a network of minicomputers that will interface with the various Johns Hopkins Hospital (JHH) clinical systems and provide aids to the clinical management of patients, radiation therapy planning and control, patient monitoring, and Cancer Center facility management (Fig. 1).

Because initial funding for the Center did not provide for all the necessary computer equipment, the activities during the reporting period were divided into three somewhat independent areas:

1. Development of an initial stand-alone system to support clinical management of patients and the Tumor Registry,
2. Procurement of a turnkey system to perform radiation therapy planning for the immediate future, and
3. Long-range planning and specification of computer requirements for the total Cancer Center system of computer aids.

SUMMARY

A Level 0 system has been defined and implemented that supports the most critical needs for patient management and some facility management functions, such as the Tumor Registry. It has been developed to operate in the APL S 360 system and is described later under the heading "Clinical Management of Patients." A second major mode of this initial system was procured as a turnkey system. The system is described later under the heading "Radiation Therapy Planning." The implementation of the two subsystems will complete the development of a Level 0 system.

The general requirements for a Level 1 system have also been defined and specified. The Level 1 system will perform the functions of the Level 0 system using a dedicated JHCC computer and will include more of the patient management functions, including interfaces with existing JHH functions. The system will be located in the Cancer Center computer room and will use existing cables to support terminals at the various work stations.

A Level 2 system was then defined that would be a natural extension of the Level 1 equipment. The Level 2 system would provide redundancy to ensure availability. It would also support additional Cancer Center functions and integrate the radiation planning and JHH central computer system as appropriate.

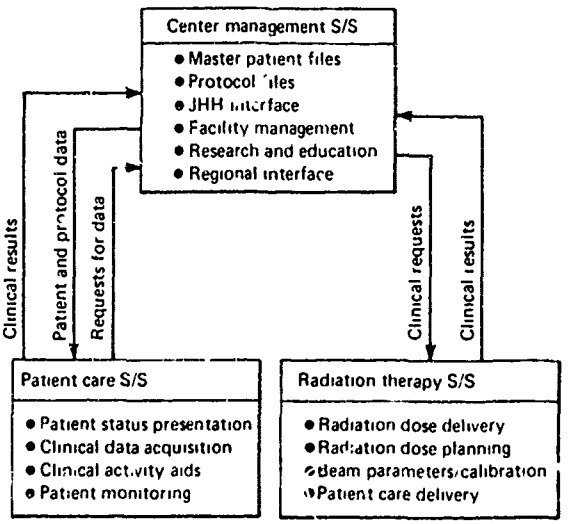

Fig 1 Functional Organization, Cancer Support System. (76-3/58)

Once a plan had been developed for the orderly growth of the JHCC computer facility, equipment for the Level 1 system was identified and budgetary estimates were prepared for equipment, clinical engineering, system development, and operational support.

DISCUSSION

Clinical Management of Patients. The Level 0 system to support the clinical management of patients was limited initially to patients treated with chemotherapy. It is based on the following aspects of patient management as carried out in the Center:

1. Patient treatment is normally directed by well defined protocols,
2. Inpatients and outpatients are subject to similar treatment plans, and
3. Emphasis is placed on the quantification of patient responses and the rapid identification of trends to enhance the timely medical response to changing clinical status.

The clinical information system is designed to support the activity by:

1. Providing an up-to-date summary of history, diagnosis, and treatment for each active patient;
2. Printing flow sheets containing major laboratory values, medications, therapy, and patient status,
3. Producing time-related plots of correlated bits of data such as blood counts and therapy, and renal, hepatic, and pulmonary function test results;
4. Printing management plans daily for each individual patient that list all protocol-directed tests and therapies plus any physician-defined modifications;
5. Printing daily work load projections for inpatient and outpatient work areas as based upon the protocol-directed activities;
6. Maintaining a flexible appointment system for outpatients appropriate to their therapy plan, and
7. Interfacing with other JHH information systems.

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JOHNS HOPKINS HOSPITAL                    HISTORY NO:
ONCOLOGY CENTER                              NAME:
                                             DATE: 10/18/76

                                  PHONE                    CLASS  JHH
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ADMITTED                DISCHARGED
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DIAGNOSIS
+-------------------------------------------------------------------+
|                                                                   |
|     JHH DIAGNOSIS       03/75                                     |
|                                                                   |
|171    MALIGNANT NEOPLASM OF CERVIX UTERI                          |
|8012.1 CARCINOMA-IN-SITU EXTENT: LOCALIZED                         |
|                                                                   |
|BASIS OF DX: PATHOLOGY                                             |
|CA. OF CERVIX                                                      |
|                                                                   |
|75-4005 - CERVIX: MARKED TO ICO WITH GLANDS, ALL QUADRANTS; PROBABLE|
|MICROINVASION IN QUADRANTS CCC MARGINS ARE NOT CLEAR.              |
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| TREATMENT                                                         |
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|  1 BX. OF CERVIX CERVICAL CONIZATION                              |
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HISTORY
+-------------------------------------------------------------------+
|                                                                   |
|FAMILY HISTORY      MOTHER ALIVE WITH CA. OF BREAST- FATHER WITH CA.|
|                    OF LARYNX                                      |
|MEDICAL HISTORY     UTI X 3, IUD X 2 MOS., PARA 1013, CHRONIC ATOPIC|
|                    DERMATITIS                                     |
|SOCIAL HISTORY      SMOKES: 1 PPD - ETOH: OCC.                     |
|ONSET OF SYMPTOMS   H/C POSITIVE PAP SMEAR                         |
|CM                  PT. DESIRES FURTHER PREGNANCIES                |
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Fig. 2 Example of Medical Abstract of Cancer Patient. (76-3/59)

The system currently provides limited on-line access via terminal; however, most functions are performed off-line in the batch mode. All programs operate on APL's computer. Transfer of these functions to a dedicated JHCC computer with considerable terminal interaction is being planned.

The implementation of the APL-operated clinical management system was divided into three phases. Phase I involved the development of an automated system for managing the JHH Tumor Registry. The registry contains a record of each JHH patient diagnosed as having cancer. The registry staff maintains one entry for each primary site diagnosed and follows up each case on an annual basis. Although the data base contains many patients not treated by the Cancer Center, all Center patients are in the data base. For this reason it was decided to automate a surrogate data base and provide a variety of reporting and searching functions.

Phase II is the expansion of Phase I to include the maintenance of full abstracts in automated form. A sample abstract is shown in Fig. 2. Abstracts are prepared for all Tumor Registry patients. For Cancer Center patients, the abstracts are enlarged to contain admission/appointment data plus specific treatment data. All items in the abstract are designed to allow searching.

The third and final phase of the APL system (Level 0) supports the management of patient treatment. For each Cancer Center patient, a

(continued)

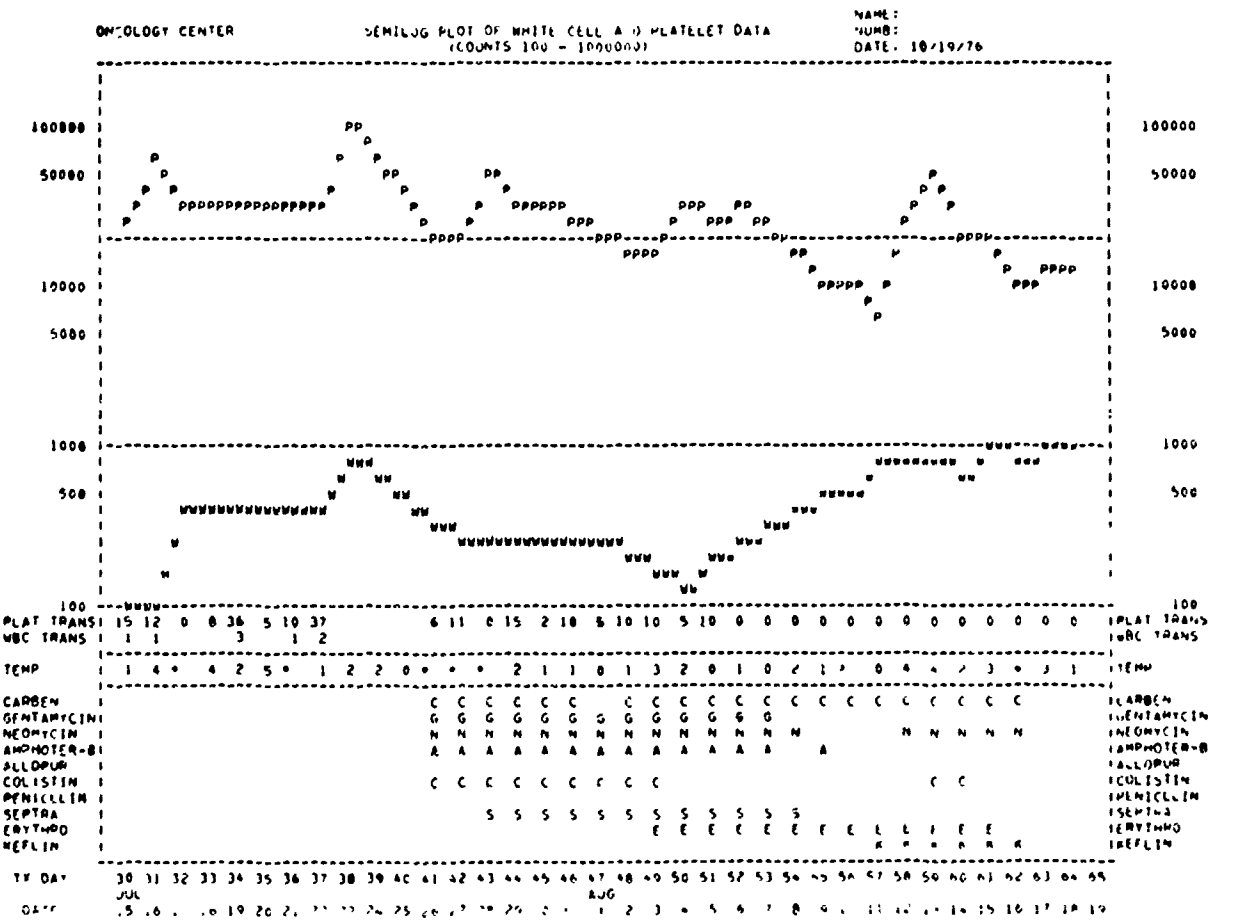

Fig. 3 Example of Data for Patient Therapy Management (76-3/60)

data base is maintained that contains an identification of the treatment protocol(s), the major laboratory test results, medications, etc. From this data base, it is possible to produce daily patient management plans and data displays designed to facilitate the decision-making process. Figure 3 illustrates an example of data for making such decisions — a time-phased plot of white cell count, temperature, and medications. Tabular outputs are available that include serial values of clinical variables, lists of hematology data, and bacteriology reports sorted by date, specimen, or organism.

Most of the Level 0 system is complete. The Phase I system has been in operation since late 1975 and contains a data base of about 20 000 patients. The Phase II system is in operation; over 1000 entries are maintained on line. Parts of the Phase III system are operational and in pilot use for the inpatients treated by the JHCC at Baltimore City Hospital.

Radiation Therapy Planning. A comprehensive survey and an evaluation of turnkey systems for radiation treatment planning were made by APL. Six systems were considered and investigated. Three were eliminated early in the evaluation because of insufficient computing speed or out-of-U.S. maintenance support. The remaining three were evaluated in depth.

The evaluation criteria included interstitial and intracavitary therapy with multiple sources, external beam planning, irregular field planning, beam-loading capability, beam retrieval, ease of contour input, speed of calculations, speed and readability of plots, cost, growth potential, and compatibility with other JHMI computer systems.

The evaluation criteria were established in conjunction with radiation oncology personnel who will use the system. Several test cases supplied by clinical radiation oncologists were run on each of the three competitive systems. A report was prepared comparing the performance of the three systems in the test cases and with the evaluation criteria. On the basis of the written report and an oral presentation, the system illustrated in Fig. 4 was selected.

Fig. 4 Turnkey System Selected for Radiation Treatment Planning. (76-3/61)

FUTURE PLANS

The JHCC will move into its new facility during October and November 1976. During the first months of transition, new Phase III capabilities will be implemented and placed in operation. Most programming activities should be complete by the end of 1976. The use of the APL computer to support the JHCC is expected to continue through 1977. With the exception of computer program maintenance activities, it is assumed that the development activity in 1977 will be dedicated to the design and implementation of a slightly expanded system that will operate on a JHCC computer and provide expanded interactive support.

Capabilities for planning radiation therapy will require extensions and additions in the future; these requirements will be addressed in the next reporting period. Only limited extention of the stand-alone turnkey system is expected; the major upgrading will occur in the Level 2 system.

Funding for the Level 1 system has been identified, and the procurement of the Level 1 system is scheduled to begin during the next reporting period. Further growth to a Level 2 system is not expected to begin until at least 1978.

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING §57
Research by: CBP
Support: Johns Hopkins Hospital, Johns Hopkins School of Medicine
J. B. Oakes and J. T. Massey
October 1975-September 1976

CLINICAL ENGINEERING

The goal of clinical engineering at the Johns Hopkins Hospital is to apply modern engineering science and technology to appropriate problems confronting the Hospital. As a result of the initial successes achieved by the Hospital's Clinical Engineering Task Force, a permanent Clinical Engineering Division within the Department of Biomedical Engineering was established in early 1972. The Division draws significant technical support from APL.

SUMMARY AND CONCLUSIONS

The Clinical Engineering Division has made several contributions to the solution of significant problems at the Johns Hopkins Hospital during the past year through the application of engineering technology. The Division's work has included a continuation of the communication study. Recent work in this area has included: (a) evaluation of effectiveness and performance of the study recommendations that have been implemented; (b) establishment of line organizations and responsibilities to provide Clinical Engineering services for the Cancer Center and the Department of Diagnostic Radiology; and (c) the establishment of a hospital-wide quality assurance program for high-technology medical devices.

In addition to these hospital-oriented efforts, the Division has continued to participate actively in the Master's Degree Program in Clinical Engineering at the Johns Hopkins School of Medicine.

DISCUSSION

The clinical engineering program at the Johns Hopkins Hospital began in 1971 with the establishment of a Clinical Engineering Task Force. Its success led to the formation of a Clinical Engineering Division within the Department of Biomedical Engineering, thus providing a permanent organization to apply engineering technology to health care problems.

Clinical engineering support for the Hospital is a major task, a summary of which is given below. In addition, the Division has played a major role in the Master's Degree Program in Clinical Engineering in the Medical School (details of the effort will be found elsewhere in this Annual Report).

Communications System Study. The major recommendations of the paging system study have been implemented. The recommendations were (a) to obtain a new frequency allocation for paging coverage throughout Baltimore as well as in the Hospital proper, (b) to purchase and install new transmitting and tone-encoding equipment, (c) to order new pocket pagers for the additional frequency, and (d) to retrofit existing pocket pagers to the new frequency. The Clinical Engineering Division will evaluate the effectiveness and performance of the new system and prepare a systems manual. Complete changeover to the new system will allow Security to have its own frequency.

Recommendations for telephone system improvement have also been implemented. These were (a) to include the addition of Centrex II features and (b) to use WATS lines to reduce long-distance telephone charges. The Division served in a consultant capacity to the Clinical Engineer of the Department of Diagnostic Radiology in designing the telephone system for the Maintenance Division. In addition, the design of the telephone service for the entire Cancer Center has been completed. The C&P Telephone Company is presently designing the telephone system for the Tower Building. The role of the Clinical Engineering Division is to act as consultant on the overall system to ensure that C&P personnel are aware of other communications media such as intercom and nurse call. During the past year, the Division has also planned a communications system for the relocated computer center.

Clinical Engineering for the Cancer Center. As reported last year, the Division aided the Director of Radiation Therapy Physics in specifying new equipment. At the present time, that equipment has been purchased and delivered, and is being installed in the new facility.

In addition, several tasks are under way that must be completed before the opening of the new Cancer Center. They include a checkout of all the cabling installed by the contractor and the wiring of cable connectors at each location. The specification, ordering, and installation of equipment for the audiovisual center in the Cancer Center Conference Room and approval to hire an engineer to begin the Clinical Engineering staff for the Cancer Center have been done. Mr. Oakes has committed 40% of his time to this program for the upcoming year.

Clinical Engineering for the Department of Diagnostic Radiology. The maintenance operation for the department has been reorganized in order to better control preventive and repair maintenance. (For example, there is currently only one man who can contact outside vendors.) In addition, Mr. Oakes is establishing a preventive maintenance and record-keeping system to evaluate the performance and effectiveness of each element in the 42-room diagnostic radiology facility. The purpose is to make maximum use of each area and procedure. Very recently a new radiation physicist for the Division of Diagnostic Radiology Physics has been hired. He will work very closely with Mr. Oakes in the

maintenance and educational responsibilities of his office.

Quality Assurance Program for the Hospital. On 29 July 1976, Dr. Heyssel, Director of the Johns Hopkins Hospital, approved the establishment of a comprehensive program to assure the safety and effectiveness of high-technology medical devices and systems used in direct patient care. The proposed plan provides for the development of a line organization with the responsibility and authority to ensure safe and effective medical devices and systems. This line responsibility has been assigned to the Clinical Engineering Division of the Department of Biomedical Engineering. The initial step in the implementation of the Quality Assurance system has been taken. Interviews have been held with each Functional Unit Director to acquaint him with requirements of the system.

Master's Degree Program in Clinical Engineering. The Clinical Engineering Program presently involves about 20 graduate students. Half are in the fourth year of the program and are taking a new course entitled "Biomedical Systems Engineering," which started in mid-September. This course and the course in Biomedical Instrumentation (which is continuing) involve APL personnel in teaching duties.

BIOMEDICAL RESEARCH, DEVELOPMENT, AND ENGINEERING § 58
Research by: CBP
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EDUCATIONAL ACTIVITIES

An important component of APL's collaborative medical program is its contribution in the area of educational activities. The main thrust of its effort within the Johns Hopkins community over the past several years has involved the Master's Degree Program in Clinical Engineering, which offers practicing engineers the opportunity for training in the engineering technology of health care delivery.

SUMMARY AND CONCLUSIONS

APL staff members have participated in several educational activities during the past year. Among these are administration of and curriculum development for the Master's Degree Program in Clinical Engineering in the School of Medicine, and continued participation in the Ph.D. seminars at the Medical School and in the Clinical Engineering educational program at George Washington University.

DISCUSSION

Educational activities are an important, integral part of APL's collaborative medical program. Although during the past year the major effort has been spent in the three general areas described below, APL has been involved in various ways with educational programs within other divisions of the University. For example, we have had evening roundtables with Dr. Talalay's M.D.-Ph.D. students and with Dr. Green's undergraduate class in Materials Science. Several of the undergraduate students in Biomedical Engineering have served internships in various projects of the collaborative biomedical program, and one student spent the summer working in the Department of Physiology.

Master's Degree Program in Clinical Engineering. This program was first offered in the fall of 1973. The curriculum requires 45 credit-hours for graduation. Phasing of the course work has been arranged so that the typical part-time student can complete the program in four years.

When the "Biomedical Systems Engineering" course was offered in mid-September, all but one of the courses within this curriculum had been designed. This course will involve a number of APL staff members both in presentations and workshops during the academic year.

Clinical Engineering Education Program at George Washington University. APL staff members have contributed to the continuing Engineering Education Program at George Washington University by participating as lecturers for several years. This year a course entitled "Design Considerations – Electrical" will be given as part of the Intensive and Coronary Care Units program.

BME-Ph.D. Program. APL staff members participated in several continuing seminar programs for Ph.D. candidates in the Johns Hopkins Medical School's Biomedical Engineering Department.